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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A01N 43/04, A61K 31/70, C07H 17/00, C07K 3/00, 13/00, 15/00, 17/00, C12N 5/00, 15/00, C12P 21/06

A1

(11) International Publication Number: WO 95/02328

(43) International Publication Date: 26 January 1995 (26.01.95)

(21) International Application Number: PCT/US94/07926

(22) International Filing Date: 15 July 1994 (15.07.94)

(30) Priority Data:

08/091,941	15 July 1993 (15.07.93)	US
08/097,354	26 July 1993 (26.07.93)	US

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(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: MAMMALIAN LOW MOLECULAR WEIGHT PHOSPHOLIPASE A2 NUCLEOTIDE AND AMINO ACID SEQUENCES

(57) Abstract

Novel mammalian phospholipase (PLA_2) nucleotide sequences and low molecular weight (about 14 KD) amino acid sequences encoded thereby are disclosed. More particularly, a cloned human $HPLA_2$ cDNA expressing $HPLA_2$-10 and its cloned rat $RPLA_2$ cDNA counterpart, expressing $RPLA_2$-10, which are characterized as PLA_2 Type IV, are disclosed. A second type of PLA_2 cDNA, characterized as PLA_2 Type III and exemplified by a rat PLA_2 cDNA encoding $RPLA_2$-8 and a partial human PLA_2 nucleotide sequence encoding $HPLA_2$-8, is disclosed. Expression of the cDNAs encode the two new types of PLA_2 enzymes which have phospholipase activity. The novel PLA_2s do not include disulfide bridges between cysteine amino acids 11 and 77 or elapid loops. However, the novel PLA_2s may include amino acid COOH-terminal extensions which can vary in length. Seventeen of the eighteen absolutely conserved amino acids in all active 14 KD PLA_2s are believed to be conserved in $RPLA_2$-8 and $HPLA_2$-8, whereas all eighteen are believed to be conserved in $RPLA_2$-10 and $HPLA_2$-10. Because the encoded sequences of $RPLA_2$-8 and $HPLA_2$-8 include only 16 cysteine amino acids, they have been designated as Type III. $RPLA_2$-10 and $HPLA_2$-10 are designated as Type IV since their encoded sequences include only 12 cysteine amino acids.

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MAMMALIAN LOW MOLECULAR WEIGHT PHOSPHOLIPASE A2 NUCLEOTIDE AND AMINO ACID SEQUENCES

This application is a continuation in part of U.S. Serial No. 08/091,941, filed July 15, 1993, entitled MAMMALIAN PHOSPHOLIPASE A_2 NUCLEOTIDE SEQUENCES AND LOW MOLECULAR WEIGHT AMINO ACID SEQUENCES ENCODED THEREBY.

Field of the Invention

The present invention relates to novel mammalian phospholipase A_2 nucleotide sequences, low molecular weight (approximately 14KD) amino acid sequences encoded thereby, clones and vectors which include the mammalian phospholipase A_2 nucleotide sequences, antisense nucleotide sequences complementary to the genes and mRNA transcripts encoding for the phospholipase amino acid sequences, nucleotide sequences having internal ribosome binding sites which allow for internal initiation of mRNA cap-independent translation, and cell lines.

Background

Phospholipase A_2s - phosphatide 2-acyl-hydrolase, EC 3.1.1.4 (hereinafter "PLA_2") constitute a diverse family of enzymes that hydrolyze the sn-2 fatty acyl ester bond of phosphogylcerides, producing

free fatty acid and lysophospholipids. See Dennis, E.A. Phospholiphases. In: The Enzymes, edited by Boyer, P. New York: Academic Press, p. 307-353 (1983). Over the past two decades, PLA_2 activities have been purified and characterized from different tissues, cultured cells, and exudates from different mammals. See Rordorf, G. et al.: J. Neuroscience, 11:1829-1826 (1991); Seilhamer, J.J. et al.: J. Biochem., 106:38-42 (1989); Langlais J. et al.: Biocham. & Biophys. Res. Comm., 182:208-214 (1992); Murakami, M. et al.: J. Biochem., 111:175-181 (1992); and Jordan, L.M. et al.: J. Chromat., 597:299-308 (1992). These enzymes have been found to vary in molecular weight, optimal pH, Ca^{2+} dependence, substrate specificity, and solubility.

To date, two classes of unrelated PLA_2s have been reported. One is a family of low molecular mass, approximately 14kDa PLA_2s which are characterized by a rigid three dimensional structure maintained by disulfide bridges and a catalytic requirement for Ca^{2+}. The other is a high molecular mass, 82kDa, intracellular PLA_2 found in the cytosolic subcellular fraction in the absence of calcium, but associated with cellular membranes at calcium concentrations from 0.1 to 10μM. See Clark, J.D. et al.: Cell, 65:1043-1051 (1991) and Sharp, J.D. et al.: J. Biol. Chem., 266:14850-14853 (1991).

In addition, several Ca^{++}-insensitive PLA_2 activities are believed to exist, however, it is also believed that as yet none of the genes encoding such activities have been cloned.

In terms of structure, low molecular weight, e.g., about 14kDa, PLA_2s rank among the best characterized enzymes. Complete primary sequences have been determined for more than 50 PLA_2s from organisms such as snakes, bees and humans. See Heinrikson, R.L.: Methods in Enzymology, 197:201-214 (1991); Davidson, F.F. et al.: J. Mol. Evolution, 31:228-238 (1990); and Dennis, E.A. Phospholiphases. In: The Enzymes, edited by Boyer, P. New York, Academic Press, p. 307-353 (1983). In all active 14kDa PLA_2s, 18 amino acids are believed to be conserved. See Heinrikson, R.L.: Methods in Enzymology, 197:201-214 (1991) and Davidson, F.F. J. Mol. Evolution, 31:228-238 (1990). Based on selected structural determinants, the low molecular weight PLA_2s have been classified into two types. See Heinrikson, R.L. et al.: J. Biol. Chem., 252:4913-4921 (1977). Type I enzymes have a disulfide bridge connecting cysteines between amino acids 11 and 77. In addition, there is an insertion of three amino acids between residues 54 and 56, the so-called elapid loop. The only identified mammalian Type I PLA_2s, see Seilhamer, J.J. et al.: DNA,

5:519-527 (1986) and Ohara, O. et al.: J. Biochem., 99:733-739 (1986), are expressed mainly in the pancreas and function extracellularly in digestion. Type II PLA_2s, on the other hand, lack the disulfide bridge between amino acids 11 and 77, have carboxy-terminal (COOH-terminal) amino acid extensions which can vary in length, but are commonly seven amino acids in length, which typically terminate in a half-cysteine joined to Cys-50 near the catalytic site His-48. The mammalian Type II PLA_2s reported to date occur in trace amounts in several tissues such as liver and spleen and are secreted from various cells in response to appropriate stimuli. See Seilhamer, J.J. et al.: J. Biol. Chem., 264:5335-5338 (1989); Kramer, R.M. et al.: J. Biol. Chem., 264:5768-5775 (1989); Komada, M. et al.: J. Biochem., 106:545-547 (1989); Kusunoki, C. et al.: Biochimica Et Biophysica Acta, 1087:95-97 (1990); Aarsman, A.J. et al.: J. Biol. Chem., 264:10008-10014 (1989); Ono, T. et al.: J. Biol. Chem., 264:5732-5738 (1988); Horigome, K. et al.: J. Biochem., 101:53-61 (1987); Nakano, T. et al.: Febs. Letters, 261:171-174 (1990); and Schalkwijk, C. et al.: Biochem. & Biophys. Res. Comm., 174:268-272 (1991). It is believed that Type II PLA_2s are associated with the pathologies of several diseases involving infection, tissue damage, and inflammation,

such as acute pancreatitis, septic shock, peritonitis and rheumatoid arthritis. See Vadas, P. et al.: Lab. Invest., 55:391-404 (1986); Pruzanski, W. et al.: Advances in Exper. Med. & Biol., 279:239-251 (1990); Uhl, W. et al.: J. Trauma, 30:1283-1290 (1990); and Malfertheiner, P. et al.: Klinische Wochenscrift, 67:183-185 (1989). Mammalian Type I and II PLA_2s share approximately 30-40% amino acid homology; however, eighteen amino acids are invariantly conserved in all known functional PLA_2s. Type I mammalian PLA_2 genes contain 4 coding exons; Type II mammalian genes contain five exons, the first of which is noncoding.

In 1990, a distinct 120 bp putative PLA_2 exon-like fragment (h10a), homologous to the amino-terminus encoding region of known PLA_2s, was obtained by screening a human genomic DNA library with a 45 bp human PLA_2 Type II oligonucleotide probe. See Johnson, L.K. et al.: Advances in Exper. Med. & Biol., 275:17-34 (1990). Zoo blots indicated that the putative exon has been highly conserved during evolution. However, additional exons indicating the presence of a complete gene, a corresponding cDNA, or evidence of transcription in different human tissues was not found.

Neuronal ceroid lipfuscinoses (NCL), or Batten disease, are terminal, inheritable, lysosomal

storage diseases of children. They are characterized by the accumulation of an autofluorescent pigment (ceroid or lipofuscin) in cells, especially neurons and epithelial pigment cells of the retina. NCL patients typically manifest high levels of the highly reactive compound, 4-hydroxynonenal. These levels are believed to be a consequence of a failure to resolve peroxidized, fatty acids in the normal way. It is believed that this failure could be the result of a phospholipase A_2 defect.

The infantile form of NCL has now been linked to chromosome 1p33-35. See Jarvela, I. et al.: Genomics, 9:170-173 (1991). The non-pancreatic PLA_2 (Type II) has also been mapped to chromosome 1. The Type II gene and two additional putative exon-like "fragments" (h8 and h10a), see Johnson, L.K. et al.: Advances in Exper. Med. & Biol., 275:17-34 (1990), are located at about 1p34 - in the middle of the region where gene for infantile NCL is believed to reside. See Jarvala, I. et al.: Genomics, 9:170-173 (1991). h8 and h10a each contain a unique sequence which is highly homologous to, but distinct from, exon two (which contains the calcium binding domain) of PLA_2 Type II.

Consequently, there is a continuing need to further identify and characterize additional PLA_2 exons if such exist. Such exons could be part of

unidentified genes. To the extent there are additional unidentified PLA_2 exons and genes, they may encode proteins (enzymes) that function in a manner different from, similar to, or overlapping with, the known PLA_2s. Moreover, such unidentified exons and/or genes and the enzymes encoded thereby may be regulated by some of the same effectors as the known PLA_2 genes and their proteins. Investigation of these unidentified exons and/or genes and their encoded enzymes may therefore result in new approaches to therapy of PLA_2-related diseases, such as Batten disease and inflammatory disease. Alternatively, these unidentified enzymes may have entirely different physiologic and pathologic functions. Thus, therapeutic approaches designed to block the activity of the known Type II PLA_2 enzymes may also block or reduce the activity of these novel enzymes, thereby producing unexpected side effects. Still further, a better understanding of the regulation of expression of the known and unidentified Type II PLA_2 genes in different tissues will likely expand the overall understanding of the biology and metabolic processes involving PLA_2s.

Summary of the Invention

In brief, the present invention overcomes certain of the above-mentioned shortcomings and drawbacks associated with the present state of the

PLA_2 art through the discovery of a novel family of mammalian PLA_2 genes or nucleic acid sequences encoding low molecular weight amino acid sequences, clones, vectors, antisense nucleotide sequences, nucleotide sequences having internal binding sites, and cell lines.

More particularly, the low molecular weight, i.e., about 14kDa, amino acid sequences encoded by the novel family of mammalian PLA_2 genes or sequences of the present invention may be generally characterized as enzymes having esterase activity specific for the acyl group at the sn2 position of glycero-phospholipids. Moreover, the novel amino acid sequences of the present invention do not include disulfide bridges between cysteine amino acids 11 and 77 and elapid loops. Still further, the novel amino acid sequences of the present invention may in some instances include COOH-terminal amino acid extensions which can vary in length. In addition, because of the difference in the number of cysteine residues in the encoded amino acid sequences, those novel PLA_2s of the present invention that include 16 cysteine amino acid residues have been designated as Type III whereas those novel Type IV PLA_2s of the instant invention include 12 cysteines and have been designated at Type IV. Exemplary of Type III PLA_2s of the present

invention are the genes identified as $RPLA_2$-8 (rat) and partial $HPLA_2$-8 (human), as well as the $RPLA_2$-8 (rat) cDNA. Examples of Type IV PLA_2s of the present invention are the cDNAs identified as $RPLA_2$-10 (rat) and $HPLA_2$-10 (human).

In accordance with the present invention, a human PLA_2-encoding cDNA, which expresses $HPLA_2$-10, see FIG. 12, has been isolated from human brain RNA by RACE-PCR technique. The $HPLA_2$-10 cDNA also has been isolated from a human stomach cDNA library. In addition, two rat PLA_2 encoding cDNAs, designated $RPLA_2$-8 (FIG. 3) and $RPLA_2$-10 (FIG. 11), have been isolated from rat brain and heart cDNA libraries, respectively. The $RPLA_2$-10 is believed to be the counterpart of the $HPLA_2$-10. $RPLA_2$-10 and $HPLA_2$-10 share about 79% and 78% homology at the open reading frame nucleic acid and amino acid sequence levels, respectively. The mature enzyme encoded by the $HPLA_2$-10 clone has a calculated molecular weight of about 13,592, whereas the mature enzyme encoded by the $RPLA_2$-8 clone has a calculated molecular weight of about 14,673. As indicated, a partial human genomic counterpart to $RPLA_2$-8, $HPLA_2$-8 genomic DNA, has been isolated. See FIG. 19.

Comparison of the $RPLA_2$-8 amino acid sequence deduced from the cDNA sequence to Type I and Type II PLA_2s is shown in FIGS. 8 and 9. The signi-

ficant structural features of the $RPLA_2$-8 protein are summarized in TABLE I. Seventeen (17) of the eighteen (18) absolutely conserved amino acids in all active 14kDa PLA_2s are conserved in $RPLA_2$-8. $RPLA_2$-8 protein does not contain either a disulfide bridge between Cysteines 11 and 77 or an elapid loop, which are both characteristic of Type I PLA_2s. $RPLA_2$-8 protein, however, does include a seven amino acid COOH-terminal extension having the sequence GRDKLHC, as shown in FIG. 27, which is a characteristic of Type II PLA_2s as evidenced in FIGS. 22 and 27. Furthermore, unlike mammalian type I and II PLA_2s which have 14 cysteine amino acid residues, $RPLA_2$-8 protein includes 16 cysteine amino acid residues. It is therefore believed that $RPLA_2$-8 encodes a novel PLA_2, which has been designated as PLA_2 Type III.

The cDNAs of $RPLA_2$-10 and $HPLA_2$-10 are 1.8kb (FIG. 11) and 1.1kb (FIG. 12), respectively. A comparison between the deduced amino acid sequences from $RPLA_2$-10 and $HPLA_2$-10 is shown in FIG. 13. FIGS. 14 and 15 are comparisons between the $HPLA_2$-10 deduced amino acid sequence and those of Type I and II human PLA_2s, respectively. FIGS. 18 and 16 are comparisons between the $RPLA_2$-10 deduced amino acid sequence and those of Type I and II rat PLA_2s, respectively. A comparison between the deduced amino acid sequences from $RPLA_2$-10 and $RPLA_2$-8 is shown in

FIG. 17. Th major structural features of human and rat PLA_2-10 deduc d amino acid sequences are listed in TABLE I. All eighteen (18) conserved amino acids in all of the active low-molecular weight, approximately 14kDa, PLA_2s are conserved in both human and rat PLA_2-10 amino acid sequences of the present invention. Like the predicted $RPLA_2$-8 amino acid sequence, human and rat PLA_2-10 amino acid sequences also lack disulfide bridges between Cys-11 and 77 and elapid loops. However, PLA_2-10 amino acid sequences are believed to differ from RPLA2-8 protein by having twelve (12) cysteine residues instead of sixteen (16). They further differ from $RPLA_2$-8 in that $RPLA_2$-10 does not have a COOH-terminal amino acid extension as depicted in FIG. 27 and $HPLA_2$-10 has only a single serine amino acid COOH-terminal extension as illustrated in FIG. 22. The PLA_2-10 proteins of the present invention have therefore been designated, as mentioned hereinbefore, as PLA_2 Type IV.

The present invention also contemplates antisense nucleotide sequences which are complementary to the genes and mRNA transcripts which encode for the Type III and Type IV PLA_2s. Exemplary of antisense sequences in accordance with the present invention are those which are complementary to the entire or portions of the nucleotide sequences set

forth in FIGS. 3, 11, 12 and 19. It should therefore be understood that the present invention contemplates any antisense nucleotide sequence which may be useful in connection with inhibiting or interfering with th expression of the Type III and Type IV PLA_2 enzyme genes and mRNA transcripts therefor.

The above features and advantages will be better understood with reference to the FIGS., Detailed Description and Examples which are set out hereinbelow. It should be understood that the biological materials of this invention are exemplary only and are not to be regarded as limitations of this invention.

Brief Description of the FIGS.

Reference is now made to the accompanying FIGS. in which are shown characteristics corresponding to the novel mammalian 14KD PLA_2s of the present invention from which certain of their novel features and advantages will be apparent:

FIG. 1 depicts a diagram of $RPLA_2$-8 cDNA showing positions of open reading frame coding region, repeats, and 5' and 3' termini (the first and last eight (8) nucleotides are cloning linkers);

FIG. 2 depicts a postulated secondary structure of $RPLA_2$-8 cDNA showing a stem and a loop containing the open reading frame coding region;

FIG. 3 depicts the $RPLA_2$-8 cDNA and derived amino acid sequence (th first and last eight (8) nucleotides are cloning linkers);

FIG. 4 depicts a diagram of the genomic DNA region containing exons 2, 3 and 4 of $RPLA_2$-8 in comparison to the corresponding cDNA;

FIG. 5 is a comparison between $HPLA_2$-8 Exon I and $RPLA_2$-8 Exon I sequences;

FIG. 6 is a comparison between $HPLA_2$-8 Exon II and $RPLA_2$-8 Exon II sequences;

FIG. 7 is a comparison between $RPLA_2$-8 Exon IV and $RPLA_2$-8 Exon IV sequences;

FIG. 8 is a comparison of $RPLA_2$-8 deduced amino acid sequence and rat PLA_2 Type I amino acid sequence;

FIG. 9 is a comparison of the $RPLA_2$-8 deduced amino acid sequence and rat PLA_2 Type II amino acid sequence;

FIG. 10 depicts a flow diagram of 3' and 5' RACE-RT PCR techniques used to obtain a full length $HPLA_2$-10 sequence cDNA from brain mRNA;

FIG. 11 depicts the $RPLA_2$-10 cDNA sequence showing primary cDNA sequence and various primer sequences, which are used in sequencing and synthesis, are underlined;

FIG. 12 depicts the $HPLA_2$-10 cDNA (Type IV) sequence and a secondary (clone $HPLA_2$10-5) cDNA

sequence which is slightly different at the 5′ end and forshortened. Various primer sequences used in sequencing and synthesis are underlined.

FIG. 13 is a comparison between deduced amino acid sequences of $HPLA_2$-10 and $RPLA_2$-10;

FIG. 14 is a comparison between $HPLA_2$-10 deduced amino acid sequence and human Type I amino acid sequence;

FIG. 15 is a comparison between $HPLA_2$-10 deduced amino acid sequence and human PLA_2 Type II amino acid sequence;

FIG. 16 is a comparison between deduced amino acid sequences of $RPLA_2$-10 and rat PLA_2 Type II amino acid sequence;

FIG. 17 is a comparison between deduced amino acid sequences of $RPLA_2$-10 and $RPLA_2$-8;

FIG. 18 is a comparison between deduced amino acid sequence of $RPLA_2$-10 and rat PLA_2 Type I amino acid sequence;

FIG. 19 depicts the partial human genomic $HPLA_2$-8 DNA sequence. Putative exon 1 and exons 2 and 4 are underlined;

FIG. 20 depicts a diagram of the vector to express discistronic mRNA. The chloramphenicol acetyl transferase and luciferase reporter genes are indicated by boxes. The intercistronic sequence that is replaced by part of $RPLA_2$-8 is shown;

FIG. 21 illustrates PLA_2 activity of expressed $HPLA_2$-10 cDNA. pCH10 is $HPLA_2$-10 cDNA cloned into an Epstein Barr virus-based expression vector. CpCH10-1B, CpCH10-1C, CpCH10-1D and CpCH20-2G are independent cell lines which express plasmid pCH10. The CpRASF-2B is a cell line which expresses plasmid pRASF into which a known human PLA_2 Type II gene has been cloned.

FIG. 22 depicts an alignment of amino acid sequences of human Types I, II and $HPLA_2$-10 PLA_2. Asterisks denote eighteen residues that have been conserved among all active PLA_2 sequences. The COOH-terminal amino acid extensions have been underscored;

FIG. 23 depicts the effects of pH on PLA_2 activity of $RPLA_2$-8 encoded enzyme (Type III). More particularly, FIG. 23 depicts the effects of pH on PLA_2 activity of $RPLA_2$-8 enzyme expressed by cell line CpR8-3'. The CpR8-3' cell line expresses plasmid pR8-3' which includes the coding region for the mature $RPLA_2$-8 protein (bases 806-1200) which is preceded by the signal peptide of pRASF (bases 131-196). Assay for PLA_2 activity is as indicated herein and in Elsbach, P. et al.: Methods in Enzymology, 197:24-31(1991);

FIG. 24 depicts the effects of calcium on PLA_2 activity of $RPLA_2$-8 encoded enzyme (Type III).

More particularly, FIG. 24 depicts the effects of calcium on PLA_2 activity of $RPLA_2$-8 enzyme expressed by cell line CpR8-3'. The CpR8-3' cell line expresses plasmid pR8-3' which includes the coding region for the mature $RPLA_2$-8 protein (bases 806-1200) which is preceded by the signal peptide of pRASF (bases 131-196). Assay for PLA_2 activity is as indicated herein and in Elsbach, P. et al.: Methods in Enzymology, 197:24-31(1991);

FIG. 25 depicts the effects of pH on PLA_2 activity of $HPLA_2$-10 encoded enzyme (Type IV). More particularly, FIG. 25 depicts the effects of pH on PLA_2 activity of PLA_2 Type II enzyme expressed by cell line CpRASF-2B and of PLA_2 Type IV enzyme expressed by cell line CpCH10-1D. The CpRASF-2B cell line expresses plasmid pRASF into which a known human PLA_2 Type II gene has been cloned. The CpCH10-1D cell line expresses plasmid pCH10 into which the $HPLA_2$-10 cDNA has been cloned. Assay for PLA_2 activity is as indicated herein and in Elsbach, P. et al.: Methods in Enzymology, 197:24-31 (1991);

FIG. 26 depicts the effects of calcium on PLA_2 activity of $HPLA_2$-10 encoded enzyme (Type IV). More particularly, FIG. 26 depicts the effects of calcium on PLA_2 activity of PLA_2 Type II enzyme expressed by cell line CpRASF-2B and of PLA_2 Type IV enzyme expressed by cell line CpCH10-1D. The

CpRASF-2B cell line expresses plasmid pRASF into which a known human PLA_2 Type II gene has been cloned. The CpCH10-1D cell line expresses plasmid pCH10 into which the $HPLA_2$-10 cDNA has been cloned. Assay for PLA_2 activity is as indicated herein and in Elsbach, P. et al.: Methods in Enzymology, 197:24-31 (1991); and

FIG. 27 depicts an alignment of amino acid sequences of rat Types I, II, $RPLA_2$-8 and $RPLA_2$-10 PLA_2s. Asterisks denote eighteen residues that have been conserved among all active PLA_2 sequences. The COOH-terminal amino acid extensions have been underscored.

Detailed Description

By way of illustrating and providing a more complete appreciation of the present invention and many of the attendant advantages thereof, the following detailed description is provided concerning the novel mammalian PLA_2 nucleotide sequences, the low molecular weight amino acid sequences encoded thereby, clones, vectors, antisense nucleotid sequences, nucleotide sequences having internal ribosome binding sites, and cell lines.

In accordance with the present invention, a 4.4 kb cDNA containing the r8 fragment, a rat genomic fragment containing sequences homologous to h8 fragment, is isolated from a rat fetal brain cDNA

library. See FIG. 1. This cDNA is about five-times larger than any mammalian PLA_2 cDNA known to date. Uniquely, the entire coding region is contained on a putative 1 kb loop flanked by 121 bp inverted perfect repeats, leaving about a 3 kb 3' "tail." See FIG. 2. The sequence of the entire cDNA is shown in FIG. 3. The size of the gene is about 15 kb. See FIG. 4. A preliminary screen of some rat tissues by reverse transcription and PCR (RT-PCR), using primers Pla8-1 and Pla8-4, reveals the pattern of $RPLA_2$-8 gene expression indicated in Table I.

TABLE I

Characteristics of Type III and IV PLA_2s

	Pre*	Pro*	Mature*
Hum Type I	MKLLVLAVLLTVAAA[1]	DSGISPR[2]	AVWQF[3]
Hum Type II	MKTLLLAVIMIFGLLQAHG[4]		NLVNF[5]
Rat Type III	MDLLVSSGMKGIAVFLVFIFC[6]	(WTTSTLS)[7]	SFWQF[8]
Hum Type IV	MKGLLPLAWFLACSVPAVQG[9]		GLLDL[10]
Rat Type IV	MKRLLTLAWFLACSVPAVPG[11]		GLLEL[12]

Human Type I PLA_2 has a 7 residue propeptide, human Type II does not. Human and rat Type IV are like Type II; Rat Type III might encode a 7 residue propeptide.

* depicts the NH_2-terminal amino acids in the amino acid sequences for the respective prepeptides, propeptides and mature peptides.

[1]represents SEQ ID NO:1:; [2]represents SEQ ID NO:2:; [3]represents SEQ ID NO:3:; [4]represents SEQ ID NO:4:; [5]represents SEQ ID NO:5:; [6]represents SEQ ID NO:6:; [7]represents SEQ ID NO:7:; [8]represents SEQ ID NO:8:; [9]represents SEQ ID NO:9:; [10]represents SEQ ID NO:10:; [11]represents SEQ ID NO:11:; [12]represents SEQ ID NO:12:.

Conserved Characteristics of Pre, Pro and Mature Peptides:

Rat Type III	Human and Rat Type IV
Phe5	Ile9
Met8	Met8
YGCYCG Ca^{2+} binding loop	YGCYCG Ca^{2+} binding loop
His48, Asp49 active site	His48, Asp49 active site
Position of Cys residues (disregarding the two extra Cys residues)	Position of Cys residues (disregarding the two missing Cys residues)

Unusual Characteristics of Pre, Pro and Mature Peptides:

Rat Type III	Human and Rat Type IV
Val9	Leu5
Two extra Cys residues	Two missing Cys residues
Ala 102, 103 missing	Ala 102, 103 missing
Unusually large variable peptide loop	

Other Characteristics of Pre, Pro and Mature Peptides:

Rat Type III	Human and Rat Type IV
No elapid loop	No elapid loops
No disulphide bridge between Cys 11 and 77	No disulphide bridges between Cys 11 and 77
Sixteen Cys residues	Twelve Cys residues
Seven COOH-terminal amino acid extension-GRDKLHC	Human Type IV-one serine COOH-terminal extension
	Rat Type IV-no COOH-terminal amino acid extension

**The numbers designating the positions for the amino acids in Table I are for th mature peptides.

Moreover, according to Northern Blot data of several tissues, a $RPLA_2$ mRNA is detected in only the testis indicating that the $RPLA_2$-8 gene is testis specific, as reported in Table II.

TABLE II

Northern blot data

Type IV (cl 10) human	
brain	-
heart	+++
kidney	-
liver	-
lung	+
pancreas	-
placenta	++
skeletal muscle	-
spleen	-
testis	-

Type IV (cl 10) rat		Type III (cl 8) rat
brain	-	-
heart	++	-
kidney	-	-
liver	-	-
lung	?	-
skeletal muscle	-	-
spleen	-	-
testis	-	++

Using parts of $RPLA_2$-8 as probes, a partial human genomic cl ne which is homologous to rat genomic clone is identified. See FIG. 19. To date, all but the third of the four exons in the human genomic DNA, see FIGS. 5-7, is identified and sequenced. The 3' flanking regions of the human and rat genes show very significant homology (about 50 percent) for about 500 bp. This conservation is unusual and suggests functional importance. It is functionally demonstrated that $RPLA_2$-8 cDNA contains an internal ribosome binding site that enables internal translation initiation.

A comparison of the significant structural features of the putative protein encoded by $RPLA_2$-8 cDNA sequence and encoded amino acid sequence to those of the corresponding pancreatic and non-pancreatic PLA_2 enzymes are shown in FIG. 8 and 9. Pancreatic PLA_2 is known as Type I and the non-pancreatic PLA_2 is designated as Type II. It is believed that PLA_2-8 encodes a novel PLA_2 which is designated as Type III. An enzyme encoded by a gene containing the h10a sequence is designated Type IV (see below). The proximity (within about a million base pair region in the mouse) of the genes for Types III and IV to the PLA_2 Type II gene suggests a common evolutionary origin as does their localization to the same band on human chromosome 1. Further, Types II,

III and IV are likely to be members of a gene family and may represent isozymes. However, a homology comparison indicates that the $RPLA_2$-8 protein is relatively distant, evolutionarily, from both Type I and Type II PLA_2 enzymes, but is believed to be probably closer to Type II.

In accordance with the present invention, human cDNA that contains the h10a fragment and rat cDNA that contains the rat counterpart are isolated. See FIGS. 11 and 12. The predicted protein sequences of $HPLA_2$-10 and $RPLA_2$-10 and comparisons to each other and Types I and II are shown in FIGS. 13-17. Some of the significant structural features of the proteins encoded by these cDNAs are shown in TABLE I. Importantly, the 18 amino acids that are believed to be requisite for PLA_2 function are conserved in both predicted proteins. See FIG. 22. This fact, plus the high degree of conservation between species, suggests that these Type IV proteins play an important role in phospholipid metabolism and processes such as membrane structuring, inflammation and intracellular signaling.

The amino acid sequences of the present invention may be produced by, for example, recombinant technology, chemical synthesis or any other methods available in the art so long as the methodology selected does not interfere with their

utilities. Likewise, the nucleotide sequences of the instant invention may be produced by, for instance, PCR technology, chemical synthesis or any other methods available in the art so long as the methodology selected does not interfere with their utilities. Moreover, amino acid residues may be deleted or added or alternative amino acid residues may be substituted for those recited in the amino acid sequences of the instant invention so long as such changes do not defeat the utilities of such amino acid sequences. Still further, it should be appreciated that the present invention contemplates any amino acid sequences which are equivalent to or constitute active fragments of the amino acid sequences for the Type III and Type IV PLA_2 enzymes of the present invention. Of course, corresponding or other changes may be made to the nucleotide sequences of the present invention to accomplish the objectives of this invention.

It should also be appreciated that the present invention contemplates a.) any antisense nucleotide sequences which are capable of inhibiting or interfering with expression of genes and mRNA transcripts encoding Type III and Type IV PLA_2 enzymes of the present invention, including any amino acid sequences that are equivalent thereto or active fragments thereof, and b.) any nucleotide sequences

having bases 116-720 of FIG. 3 and any equivalent fragments thereto or active fragments thereof that allow for internal initiation of mRNA cap-independent translation. Like other nucleotide sequences of the present invention, substitutions, deletions and additions may be made to the antisense nucleotide sequences and the nucleotide sequences having internal ribosome binding sites of the present invention so long as the objectives of the present invention are not defeated.

$\underline{HPLA_2\text{-}10}$

In order to clone an cDNA containing the putative $HPLA_2$ exon, two primers, HClo10-1 and HClo10-1a, are generated according to the 120 bp presumptive exon II sequence. See FIG. 12. PCR amplification with these primers is used to screen human child brain, adult brain, liver, heart, and various white cell cDNA libraries. PCR amplification products are not obtained.

Since zoo blots have indicated that this putative exon is evolutionarily conserved, a rat genomic cosmid library (Clontech, Inc.) is screened using a PCR-generated copy of the HClo10-1 - HClo10-1a fragement as a probe. Three unique positive clones are identified. Southern blot anaysis of the three EcoRI-digested clones using the HClo10-1 - HClo10-1a fragment as a probe identifies a

common 5kb band. This band is subcloned into EcoRI-digested pUC13 and sequenced. A region (rat-10 putative exon II) in the 5 kb sequence highly homologous to h10a is identified by computer analysis.

In order to search for the presence of exon I, the 5kb human genomic DNA clone containing putative exon II is sequenced completely. Computer analysis of the sequence identified two highly homologous regions. One appears to be exon II. It contains a consensus splice acceptor site at its 5′ end and a consensus splice donor site at its 3′ end. The other region, located about 1.2 kb 5′ of the exon II, contains a consensus splice donor site at its 3′ end and a putative in-frame ATG start codon at its 5′ end. It is likely to be exon I. Furthermore, when these two putative exons are joined together using the assumed splice donor and acceptor sites, the resulting sequence encodes a signal peptide and 41 amino acids which have significant homology to the amino terminus of known, mature PLA_2s.

After determining the putative exon I sequence, H10-A, a 5′ primer located within exon I, and H10-1a, a 3′ primer located within exon II, see FIG. 12, are used for RT-PCR of total human brain and lymphoblast RNA. A unique 140bp band from both PCR reactions is sequenced. The 140 bp contains coding exons I and II, but not the putative intron I of

$HPLA_2$-10. 5' and 3' RACE-RT PCR techniques, Frohman, M.A. et al.: PNAS, 85:8998-9002 (1988); O'Hara, O. et al.: PNAS, 86:6883-6887 (1989); and Loh, Y. et al.: Science, 243:217-220 (1989), are then used to generate the full length cDNA sequence from total human brain RNA. See FIG. 10. The entire cDNA sequence, designated $HPLA_2$-10, is shown in FIG. 12. Exon-intron junction sites are determined by genomic DNA analysis. Since the genomic DNA clone containing the first 120 bp of $HPLA_2$-10 is not obtained, it has not been determined if there are any introns in this region of the $HPLA_2$-10 genomic sequence. If no additional exons are found, $HPLA_2$-10 will apparently have an exon-intron structure typical of known Type II PLA_2s with a 5' untranslated exon followed by four protein coding exons.

Primers H10-A (bases 149-170) and H10-C (bases 520-548) are used to screen by PCR amplification a human stomach cDNA library (Clonetech, Inc.). A 399 bp and a 290 bp PCR amplification product are obtained only from the stomach cDNA library. The two PCR fragments are cloned into pUC19 and sequenced. The sequence of the 399 bp fragment is identical to the $HPLA_2$-10 RACE-RT PCR generated cDNA sequence from bases 148 to 541. The 290 bp fragment is identical to the 399 bp fragment except that it is missing bases 316 to 422

which encompass the 5′ end of exon III. See FIG. 11. The same two PCR fragments are also amplified from total human brain and lymphocyte RNA using primers H10-A and H10-C. The 290 bp PCR product is much less abundant than the 399 bp product when amplified from human stomach and brain RNA and stomach cDNA library. Since the 290 bp product codes only for the signal peptide and the first 41 amino acids of the mature protein because of an in-frame stop codon immediately following the 41st amino acid, the in vivo significance of this product is unknown at this time.

Using the 399 bp PCR product as a probe, $6x10^5$ individual plaques from the human stomach cDNA library are screened. Four positive clones are identified. The clones, designated $HPLA_2$-10-2, -3, -5, -7, have inserts of 1.4, 2.3 0.9, and 0,8 kb, respectively. The inserts of these clones are released by EcoRI digestion, subcloned into pUC19 and sequenced completely. $HPLA_2$-10-2 contains exon I-intron I-exon II of $HPLA_2$-10; $HPLA_2$-10-3 contains intron III-exon IV-intron IV of $HPLA_2$-10. The sequences of both $HPLA_2$-10-5 and $HPLA_2$-10-7 are identical to the corresponding regions of the RACE-RT-PCR generated $HPLA_2$-10 sequence except that the 5′ end of the $HPLA_2$-10-5 starts at base 142 of

the RACE-RT-PCR sequence and the 5′ end of $HPLA_2$-10-7 starts at base 23.

To determine the transcription pattern of $HPLA_2$-10, a Human Multiple Northern Blot (Clontech, Inc.) is probed with a 399 bp fragment, i.e., $HPLA_2$-10 PCR probe, generated by PCR with primers H10-A (bases 149-170) and H10-C (bases 520-548). As seen in TABLE II, a 1.2 kb transcript is detected in heart and, less abundantly, in liver and lung RNA. In addition, a 2 kb transcript is detected in placental RNA. This suggests that the expression of $HPLA_2$-10 is not only tissue specific, but that alternative forms of the protein may be expressed in different tissues. The 2 kb transcript seen in placental RNA may result from the use of a different promoter, alternative splicing or the use of an alternative poly A site.

The $HPLA_2$-10 cDNA encodes a mature protein of about 118 amino acids with a calculated molecular mass of about 13,592 Daltons. The amino acid sequence has significant homology to known PLA_2s. All of the 18 invariantly conserved amino acids in known active low molecular weight PLA_2s, see Davidson, F.F.: J. Mol. Evolution, 31:228-238 (1990), are conserved in this novel protein. See FIG. 22. However, $HPLA_2$-10 contains neither the disulfide bridge between Cys 11 and 77 nor the elapid loop

characteristic of Type I PLA_2s. $HPLA_2$-10 does, however, c ntain a single serine amino acid COOH-terminal extension, as shown in FIG. 22, which is more characteristic of a Type I than Type II PLA_2. As depicted in FIG. 22, Human Type I has a two amino acid COOH-terminal extension whereas Human Type II has a seven amino acid COOH-terminal extension. Furthermore, unlike mammalian Types I and II PLA_2s which have 14 cysteine residues, this putative $HPLA_2$ only has 12. The overall homology between $HPLA_2$-10 and a consensus Type I PLA_2 is about 30.5% while the overall homology between $HPLA_2$-10 and a consensus Type II PLA_2 is about 40.6%. The predicted isoelectric point (pI) of this protein is about 6.2 while that of other known Type II PLA_2s is about 10.5.

To test whether this $HPLA_2$-10 gene encodes an active, secreted PLA_2, an Epstein Barr virus-based expression vector (pCEP) is used to express the $HPLA_2$-10 cDNA in human 293s cells. pCEP contains two regions of the EBV genome required for episomal maintenance (EBNA-1 and OriP), a drug resistance gene for selection in human cells (hyg), bacterial sequences for maintenance in E. coli, a drug resistance gene for selection in E. coli (amp), and an expression cassette for the production of high levels of mRNA from an introduced sequence by using an Rous/Sarcoma virus long terminal repeat (RSV LTR)

promoter and an Simian virus 40 (SV40) polyadenylation signal. $HPLA_2$-10-5', a 5' primer beginning at base 126 of $HPLA_2$-10 and containing a 10 nucleotide NheI linker at its 5' end, and $HPLA_2$-10-3', a 3' primer ending at base 555 and beginning with a 10 nucleotide XhoI linker, are used for reverse-transcriptase-polymerase chain reaction (RT-PCR) of total human brain RNA to generate the appropriate cDNA insert. The PCR product is blunt-end ligated to HincII-digested pUC19 and sequenced. The insert is then released by digestion with NheI and XhoI and is cloned into the NheI-XhoI sites of pCEP. The resulting plasmid is designated pCh10.

A known human Type II PLA_2 cDNA is cloned into pCEP for use as a positive control. PCR primers RASF-5' and RASF-3' are used to amplify bases 130 to 581 of pRASF, a plasmid containing the entire human known PLA_2 Type II cDNA. See Seilhamer, J.J.: J. Biol. Chem., 264:5335-5338 (1989). The resulting plasmid is designated pRASF and is used as a control. The $HPLA_2$-2B (Type II) enzyme, as depicted in FIGS. 25 and 26, are expressed by pRASF and used as a control.

Purified plasmid DNA is transfected into human 293s cells which are selected in DMEM containing 200 ug/ml hygromycin. Medium samples from

multiple cell lines transfected with either pCH10, pR8-3′ or pRASF are then assayed for PLA_2 activity. See FIG. 21. PLA_2 activities derived from cell lines transfected with plasmids pCH10, pR8-3′, and pRASF are accumulated in the medium. Neither 293s cells nor multiple cell lines transfected with an unrelated PLA_2 cDNA inactivated by a one base pair deletion at the 5′ end of the mature protein show detectable PLA_2 activity in the medium even after 72 hours. Cell lysates that are prepared by sonication from cells stably transfected with either pCH10 or pRASF show approximately 50% of the activity of 72 hour medium samples.

Two cell lines, CpCH10-1D expressing pCH10 and CpRASF-2B expressing pRASF, are chosen for comparative study. The pH profile for the enzyme expressed by the cell lines is shown in FIG. 25. PLA_2 activity of $HPLA_2$-10 starts at about pH 5 and significant activity is reached at between about pH 6.5 and about pH 7.5 and remains relatively steady up to at least about pH 9.5, whereas the control Type II PLA_2 reaches peak activity at between about pH 7.0 and about pH 7.5 and then progressively declines.

Calcium concentration versus enzyme activity profiles for CpCH10-1D and CpRASF-2B are shown in FIG. 26. $HPLA_2$-10 appears to be a calcium-dependent PLA_2 having activity starting at

about 0.07 mM Ca^{2+} amd reaching maximal activity at between about 7 mM and about 100 mM Ca^{2+}. The activity of $HPLA_2$-10 then slowly decreases, but maintains significant activity, as the Ca^{2+} concentration approaches about 500 mM or more. This profile differs from that of the control cell line CpRASF-2 (Type II PLA_2) which shows maximal activity at between about 0.5 mM and 3.0 mM Ca^{2+} and becomes inactive at Ca^{2+} concentrations at about 100 mM or greater. Since $HPLA_2$-10 expresses at least half of its maximal activity at Ca^{2+} concentrations between 1 and 100 mM, similar to previously described Type II phospholipases, see Marshall: Biochemical Pharmacology, V. 44:1849-1858 (1992), it is likely that $HPLA_2$-10 is capable of functioning at concentrations found intracellularly (0.1 to 2 μM) and extracellularly (1mM).

$RPLA_2$-8

Two PCR primers, Pla8-1 and Pla8-2 (FIG. 3), are generated using the reported rat r8 presumptive exon II sequence. See Seilhamer, J.J. et al.: J. Cell. Biochem., 39:327-337 (1989). Four size-fractionated, newborn rat brain cDNA λZAPII libraries (two 0.5-1.5kb, one 1.5-4kb, and one greater than 4kb, provided by Dr. L. Yu, Indiana School of Medicine, are directly amplified by PCR, See Friedman, K.D. et al.: Nucleic Acids Research;

16:8718 (1988), using primers pla8-1 and pla-2. Only the)4 kb insert library gives the proper size 120 bp fragment prediced by the Clo8 DNA sequence. The band is purified from an agarose gel using a QIAEX gel extraction kit (QIAGEN), cloned into m13mp18, and is sequenced using a Sequenase kit (USB). The sequence data confirms the proper identity of the PCR product. A total of 10^6 individual clones from the cDNA library are screened using the PCR product as a probe. Only two clones hybridize. The restriction maps of the two clones are believed to be identical. One of them, clo8-2, is sequenced completely. The sequence, designated RPLA2-8, is shown in FIG. 3.

$RPLA_2$-8 is a 4.4kb cDNA, which is about five-times larger than any known mammalian 14kDa PLA_2 cDNA. See Seilhamer, J.J. et al.: DNA, 5:519-527 (1986); Seilhamer, J.J. et al.: J. Biol. Chem., 264:5335-5338 (1989); Ohara, O. et al.: Proc. Natl. Acad. Sciences U.S.A., 86:6883-6887 (1989); Kramer, R.M. et al.: J. Biol. Chem., 264:5768-5775 (1989); and Komada, M. et al.: J. Biochem., 106:545-547 (1989). The 480 bp coding region is believed to be contained in a putative 1.2kb loop flanked by 121 bp perfect inverted repeats. See FIG. 2. This stem-loop is flanked by perfect 121 bp inverted repeats. This stem-loop structure leaves about 3kb of 3′ "tail." See FIGS. 1 and 2. Translation of

RNAs containing such a secondary structure cannot readily be explained by the conventional translation scanning model. See Pain, V.M.: Biochemistry J., 235:625-637 (1986). Nevertheless, it is believed that there is an internal ribosome binding site between the 5' repeat sequence and ATG translation start site. Cloning the sequence between base 116 and 720, see FIG. 3, in both normal and reverse orientations in front of an internal luciferase gene which lies downstream of a CAT gene, see Macejjak, D.G. et al.: Nature, 353:90-94 (1991), see FIG. 20, followed by detecting luciferase gene expression in transfected Hela cells (with positive and negative control constructs), confirms that the fragment does contain a internal ribosome binding sequence. Luciferase expression is significantly higher when the fragment is cloned in normal orientation then in reverse orientation. It is believed that the translation of mRNAs initiated by an internal ribosome binding mechanism may play an important role in mitosis, meiosis or specific viral infection, because cap-dependent translation during mitosis in mammalian cells is unlikely, due to the presence of underphosphorylated and therefore nonfunctional translation initiation factor, eif-4F. See Macejjak, D.G. et al.: Nature, 353:90-94 (1991). It is

therefore believed that the $RPLA_2$-8 gene product could play a role during these processes.

As a preliminary study, the pattern of $RPLA_2$-8 gene expression, see TABLE III, is examined by screening rat tissues with reverse transcription followed by PCR (RT-PCR), using primers pla8-1 and pla8-2. See FIG. 3.

TABLE III

Reverse Transcription-PCR (RT-PCR) of Total RNA of Different Rat tissues by Primers Clo8-1 and Clo8-1a

1.	Brain	+
2.	Cerebellum, Brain Stem	+
3.	Kidney	+
4.	Lung	+
5.	Heart	+
6.	Muscle (?)	+
7.	Pancreas	-
8.	Small intestine	-
9.	Liver	-
10.	Prostate	-
11.	Bladder	-
12.	Spleen	-
13.	Adrenal	-
14.	Submaxillary	-

In addition, to determine transcription patterns of $RPLA_2$-8 and $RPLA_2$-10, a Rat Multiple Northern Blot (Clontech, Inc.) is probed with a 489 bp fragment, i.e., $RPLA_2$-8 PCR probe, generated by PCR with primers RClo8-5' (bases 716-742) and Rclo8-3' (bases 1178-1205). A rat Multiple Northern Blot (Clontech, Inc.) is also probed with a 427 bp fragment, i.e.,

$RPLA_2$-10 PCR probe, and amplified using primers Rclo10-5′ (bases 226-253) and Rclo10-3′ (bases 627-653). As seen in TABLE II, an $RPLA_2$-8 mRNA is detected in testis only and an $RPLA_2$-10 mRNA is detected in heart and perhaps lung only.

In order to determine the exon-intron junction sites and confirm the 121 bp direct repeat sequence in the genomic DNA, a 15 kb rat genomic DNA clone containing $RPLA_2$-8 coding exon II is analyzed by Southern blot, and partial sequencing. The 15 kb genomic DNA structure is shown in FIG. 4. It does not contain exon I and the 5′ 121 bp repeat, but it does contain the 3′ 121 bp repeat. To further investigate the 5′ rat genomic DNA sequence, a cosmid genomic DNA library (Clontech, Inc.) is screened using a PCR-generated fragment containing RPLA2-8 exon I-intron I-exon II. Twelve positive clones are indentified. Restriction mapping indicates that all clones (about 40 kb each) are identical. Unfortunately, the cosmid clones could not contain the 5′ 121 bp repeat because their 5′ ends are located in intron I. Thus, RT-PCR is used to confirm the presence of the 5′ 121 bp direct repeat sequence. Pla8-7, a 22 bp 5′ primer starting at base 73, which lies within the 121 bp repeat sequence and pla8-8, a 22 bp 3′ primer ending at base 212, see FIG. 3, are generated to conduct RT-PCR of rat brain total RNA.

The resulting RT-PCR fragment is purified from the agrose gel and cloned into m13mp18, and the sequence is confirmed to be as predicted by the cDNA.

To test whether this PLA_2-8 gene encodes an active, secreted PLA_2, an Epstein Barr virus-based expression vector (pCEP) is used to express the $RPLA_2$-8 cDNA in human 293s cells. pCEP contains two regions of the EBV genome required for episomal maintenance (EBNA-1 and OriP), a drug resistance gene for selection in human cells (hyg), bacterial sequences for maintenance in E. coli, a drug resistance gene for selection in E. coli (amp), and an expression cassette for the production of high levels of mRNA from an introduced sequence by using an Rous/Sarcoma virus long terminal repeat (RSV LTR) promoter and an Simian virus 40 (SV40) polyadenylation signal. pR8-3', a chimeric construct, is constructed as follows. RASF-5', a 5' primer beginning with a 10 nucleotide NheI linker followed by 22 nucleotides starting at base 130, and Ju9, a 22 nucleotide 3' primer complementary to base 177 and 198, see Seilhamer, J. et al.: J. Biol. Chem., 264:5335-5338 (1989), are used to PCR amplify plasmid pRASF from bases 130 to 198. pRASF contains the entire known PLA_2 Type II cDNA. See Seilhamer, J. et al.: J. Biol. Chem., 264:5335-5338 (1989). The PCR product is purified and is digested with NheI

plus NcoI. JuR8-11, a 5' primer with a total length of 31 nucleotides, beginning with GCCATGGGA followed by base 806 to 827 of $RPLA_2$-8 sequence, see FIG. 3, and R8-3', a 3' primer starting with a 10 nucleotide NheI linker at its 5' end, followed by 22 nucleotides complementary to $RPLA_2$-8 base 1178 to 1200, see FIG. 3, are used to PCR amplify plasmid $RPLA_2$-8. The PCR product is purified and digested with XhoI plus NcoI. Both digested PCR products are then ligated together into XhoI-NheI digested pCEP. Sequencing is carried out to confirm the nucleotide sequence of pR8-3'. CpR8-3' is a single clone of cells chosen to represent the typical pH optimum and Ca^{++} dependence of CpR8 transfected 293s cells. The effects of pH and calcium concentration on enzyme activity are illustrated in FIGS. 23 and 24, respectively, for the $RPLA_2$-8 enzyme (Type III) and are similar, but different to the pH and calcium profiles for the $HPLA_2$-10 enzyme (Type IV) encoded for by the $HPLA_2$-10 cDNA cloned into plasmid cPH10, as shown in FIGS. 25 and 26, respectively. In other words, $RPLA_2$-8 also appears to be a pH and calcium-dependent PLA_2 enzyme having activity starting at about pH 5.5 and having significant activity at between about pH 7 and about pH 9 and having activity starting at about 0.1 mM Ca^{2+} and having significant activity at between about 0.3 mM and about 2 mM Ca^{2+}, respectively. The

activity of $RPLA_2$-8, however, apparently progressively declines at a pH of greater than about 9 and at a calcium concentration of greater than about 2 mM. Nonetheless, FIGS. 23-26 illustrat phsopholipase activity for the Type III and Type IV phospholipase enzymes of the present invention. Moreover, FIGS. 23-26 show that the pH and calcium profiles for the Type III and Type IV phospholipase enzymes of the present invention are different from the pH and calcium profiles for phospholipases known heretofore.

It should be appreciated by those skilled in the art that the novel PLA_2 Type III and Type IV enzymes described in the instant application may have many different potential uses.

Although both "Type II" soluble PLA_2 and intracellular membrane-associated PLA_2 have been shown to mediate many aspects of the inflammatory cascade, it may well be that the new PLA_2 enzymes may also play a role, either by directly functioning to liberate arachidonic acid and 2-lysophospholipid, or by replacing the functions of the former in tissues and/or individuals in which the enzymes may be otherwise missing. As such, inhibition of these new enzymes by standard strategies known in the art (e.g., crystallography-based rational drug design;

antisense; triple helix; monoclonal antibodies) could be valuabl in anti-inflammatory therapy.

Phospholipases A_2 are involved in other processes vital to sustaining life in humans, including but not limited to pulmonary surfactant turnover, biomembrane maintenance and metabolism, various lipid catabolic pathways, platelet activation factor metabolism, and sperm-mediated egg activation. First, it is possible that certain diseases present today involve alterations in these functions, and could be treated therapeutically with exogenously added recombinant PLA_2 or anti-PLA_2. Second, as new PLA_2-inhibiting anti-inflammatory therapeutics are developed, many may exhibit cross-inhibition with these other new enzymes, thereby causing undesired side-effects. Both knowledge of the sequence/structure of these new enzymes, and the ability to restore their function through addition of the appropriate recombinant enzyme could be of value in reducing such side-effects.

Although these enzymes have been characterized as PLA_2 enzymes, they may well have other vital enzymatic activities. For example, LCAT (lecithin-cholesterol acyl transferase) also exhibits PLA_2 activity. Alternatively, these enzymes may function as phospholipases A1, phospholipases B,

phospholipases C, lysophopholipases, acyl hydrolases, ribonucleases, lipases, or ph sphodiesterases, all of which are esterases which resemble phospholipase A_2 in chemical activity. If this is the case, these new enzymes could be used to treat defects in a variety of metabolic pathways.

PLA_2 is also useful in the food processing industry. See Dutilh et al.: J. Sci. Food Agricul., 32:451-458 (1981), and in the preservation of fish, see Mazeaud et al.: J. Fish Res. Board Cun., 33:1297-1303 (1976). Recombinant forms of the instant new PLA_2s may be useful to replace natural sources of these enzymes.

$RPLA_2$-8, by virtue of its specific synthesis in rat testis, may play a key role in activation during fertilization by sperm. Therefore, antagonism of its function may prove useful as a specific anti-fertility reagent in pests such as rodents.

$HPLA_2$10 and $RPLA_2$-10, by virtue of their specific synthesis in cardiac tissue, may play a key role in cardiac lipid metabolism specific to cardiac tissue, and may indicate a specialized new function for this enzyme. A major component of heart tissue is cardiolipin, and Type IV phospholipase may mediate metabolism of this related diphospholipid in this organ. Therefore, recombinant forms of the new PLA_2s

could prove useful in the treatment of disorders involving cardiac phospholipid metabolism.

In addition, the new PLA_2s have been mapped into a genetic locus known to be associated with Batten's disease (or Neuronal Ceroid Lipfuscinosis; NCL). Since the latter disorder has been shown to involve alterations in activity of certain phospholipases, see Dawson et al.: Advances in Experimental Medicine & Biology, 266:259-270 (1989), these new enzymes may be useful as a therapeutic to treat the former, and as a diagnostic to detect the presence of these genetic abnormalities so that proper counseling and early treatment of the disease would be possible.

Examples of various embodiments of the present invention will now be further illustrated with reference to the following Examples.

Example I - CpCH10-1D Cell Line Transfected with pCH10 which Expresses $HPLA_2$-10

Total RNA is prepared according to the method of Chomcyzmski and Sacchi: Analytical Biochemistry, 162:156-159 (1987). 5' and 3' RACE-RT PCR techniques are used to generate the full length cDNA from total human brain RNA as described by Ishisaki: Biochem. Biophysic. res. Comm., 162:1030-1036 (1989), and outlined in FIG. 10. PCR amplifications are done using 30 cycles at 95°C for 20 seconds, 60°C for 20 seconds and 72°C for 75

seconds in 100 µl of buffer containing a final concentration of 1.5 mM $MgCl_2$, 200 µM dNTP, 100 mM Tris-HCl, pH 8.3, and 3 units Taq polymerase. Anchor (300 ng) and adaptor (50 ng) primers are used in both 5' and 3' RACE-RT PCR. Primers H10-C (300 µg) and H10-1a (300 µg) are used for 5' RACE-RT PCR. Primers H10-A (300 µg) and H10-1 (300 µg), see FIG. 10, are used for 3' RACE-RT PCR. Primer sequences are listed in TABLE IV.

TABLE IV

Primers	Sequences
H10-A	CTGGCTTGGTTCCTGGCTTGTA[13]
H10-1	GCAAGGAGGCTTGCTGGACCTA[14]
H10-1a	ATCGGTGCCATCCTTGGGGGTT[15]
H10-C	GCAGAGGATGTTGGGAAAGTAT[16]
H10-5'	GAATTCGCTAGCCAGAGATGAAAGGCCTCCTCCCACTGGCTTGG[17]
H10-3'	CTCGCTCTCGAGGCCCTAGGAGCAGAGGATGTTGGGAAA[18]
Anchor	GGCCACGCGTCGACTAGTAC(T)[19] 17
Adaptor	GGCCACGCGTCGACTAGTAC[20]

[13]represents SEQ ID NO:13:; [14]represents SEQ ID NO:14:; [15]represents SEQ ID NO:15:; [16]represents SEQ ID NO:16:; [17]represents SEQ ID NO:17: [18]represents SEQ ID NO:18:; [19]represents SEQ ID NO:19:; [20]represents SEQ ID NO:20:.

$6x10^5$ clones from a human stomach cDNA phage library (Clontech, Inc.) and $5x10^5$ clones from a rat genomic DNA cosmid library (Clontech, Inc.) are screened according to the procedures provided by Clontech Inc.

A Human Multiple Northern Blot (Clontech, Inc.) is hybridized according to the manufacturer's directions.

293s cells (ATCC CRL 1573) are grown in Dulb cco's modified Eagle's medium (DMEM)

supplemented with 10% fetal bovine serum. Approximately $7.5x10^5$ cells are transfected with 10 µg of purified supercoiled plasmid DNA from either pCH10 or pRASF to create cell lines of the type CpCH10-1D and CpRASF-2B, respectively, according to the methods of Kingston, R.E.: Calcium Phosphate Transfection in Current Protocols in Molecular Biology. ed. Frederick M. Ausubel et al., pp. 9.1.1-9.1.3 (1989). Twenty-four hours after transfection, 200 units per ml of hygromycin is added to the medium. Stably-transfected, hygromycin-resistant colonies are selected ten days after transfection and are maintained in DMEM containing 200 units per ml of hygromycin. To test for PLA_2 activity, $2.0x10^6$ cells are plated in a 25 cm^2 flask and medium is collected 24, 48 and 72 hours after plating.

Autoclaved [$1-^{14}C$] oleic acid-labeled Escherichia coli (E. coli) JM109 is prepared according to the methods described by Elsbach, P. et al.: Methods in Enzymology, 97:24-31 (1991) for use as a PLA_2 substrate. Briefly, 20 µl medium is incubated for 15 minutes at 37°C with E. coli substrate (a mix of $2.5x10^8$ labeled and unlabeled bacteria to provide 10,000 cpm) in a total volume of 250 µl (40 mM Tris/HCl, pH 7.8, 150 mM NaCl, 10 mM Ca^{2+}). The reaction is stopped by the addition of

250 µl ice cold 0.5% (W/V) fatty acid-poor BSA (USB). After incubation on ice for 5 minutes, the samples are centrifuged at 10,000 x g for 3 minutes and 250 µ 1 of the supernatant containing released [1-^{14}C]oleic acid is counted in a scintillation counter.

The pH optimum for human Type IV PLA_2 enzyme activity is determined using 20 µl of medium diluted to produce approximately 10% substrate hydrolysis. Sodium acetate buffer (final concentration 25 mM) is used for the pH range 4-6.5 and Tris/HCl buffer (final concentration 25 mM) for the pH range 7-9. See FIG. 25.

The calcium dependence of the human Type IV enzyme activity is examined in the calcium concentration range 0-400 mM. The buffer solution (Tris/HCl, pH 7.5, final concentration 25 mM) is prepared with doubly distilled, deionized water which contained a minimal amount of metal ions. EDTA (300 mcM) is added to the assay mixture in order to chelate the residual calcium. 20 µl of medium is diluted to produce 10% substrate hydrolysis. See FIG. 26.

Example II - CpR8-3'Cell Line Transfected With pCR8 Which Epxresses $RPLA_2$-8

293s cells (ATCC CRL 1573) are grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum.

Approximately $7.5x10^5$ cells are transfected with 10 µg of purified supercoiled plasmid DNA from pR8-3' to create a cell line of the type CpR8-3' according to the methods of Kingston, R.E.: Calcium Phosphate Transfection in Current Protocols in Molecular Biology. ed. Frederick M. Ausubel et al., pp. 9.1.1-9.1.3 (1989). Twenty-four hours after transfection, 200 units per ml of hygromycin is added to the medium. Stably-transfected, hygromycin-resistant colonies are selected ten days after transfection and are maintained in DMEM containing 200 units per ml of hygromycin. To test for PLA_2 activity, $2.0x10^6$ cells are plated in a 25 cm^2 flask and medium is collected 24, 48 and 72 hours after plating.

Autoclaved [1-^{14}C] oleic acid-labeled Escherichia coli (E. coli) JM109 is prepared according to the methods described by Elsbach, P. et al.: Methods in Enzymology, 97:24-31 (1991) for use as a PLA_2 substrate. Briefly, 20 µl medium is incubated for 15 minutes at 37°C with E. coli substrate (a mix of $2.5x10^8$ labeled and unlabeled bacteria to provide 10,000 cpm) in a total volume of 250 µl (40 mM Tris/HCl, pH 7.8, 150 mM NaCl, 10 mM Ca^{2+}). The reaction is stopped by the addition of 250 µl ice cold 0.5% (W/V) fatty acid-poor BSA (USB). After incubation on ic for 5 minutes, the

samples are centrifuged at 10,000 x g for 3 minutes and 250 µ 1 of the supernatant containing released (1-^{14}C]oleic acid is counted in a scintillation counter.

The pH optimum for human Type III PLA_2 enzyme activity is determined using 20 µl of medium diluted to produce approximately 10% substrate hydrolysis. Sodium acetate buffer (final concentration 25 mM) is used for the pH range 4-6.5 and Tris/HCl buffer (final concentration 25 mM) for the pH range 7-9. See FIG. 23.

The calcium dependence of the human Type III enzyme activity is examined in the calcium concentration range 0-400 mM. The buffer solution (Tris/HCl, pH 7.5, final concentration 25 mM) is prepared with doubly distilled, deionized water which contained a minimal amount of metal ions. EDTA (300 mcM) is added to the assay mixture in order to chelate the residual calcium. 20 µl of medium is diluted to produce 10% substrate hydrolysis. See FIG. 24.

Example III - PLA_2 Activity

7.5 x 10^5 293s cells are transfected with 10 ug of supercoiled plasmid DNA according to the method of Kingston, R.E.: Calcium Phosphate Transfection in Current Protocols in Molecular Biology. ed. Frederick M. Ausubel et al., pp.

9.1.1-9.1.3 (1989). Hygromycin-resistant colonies are selected 10 days after transfection and ar maintained in DMEM containing 200 units of hygromycin. CpCH10-1B, CpCH10-1C, CpCH10-1D and CpCH10-2G are independent, hygromycin-resistant cell lines transfected with pCH10, a plasmid containing the human Type IV PLA_2 cDNA; CpRASF-2B is a hygromycin-resistant cell line transfected with pMCH6, a plasmid containing the known Type II PLA_2 gene. CpR8-3' is a hygromycin-resistant cell line transfected with pR8-3', a plasmid containing the rat Type III PLA_2 cDNA. These cell lines are tested two months after their stable transfection. Each has been maintained and subcloned in hygromycin-containing medium. For this experiment, exponentially growing cells are plated at 4 x 10^5 cells per ml. Medium samples are taken 24, 48 and 72 hours after plating. 20 µl of each medium sample is assayed under standard conditions, see Elsbach, P. et al.: Methods in Enzymology, 197:24-31 (1991) for PLA_2 activity. Activity is expressed as a fraction of autoclaved [1-^{14}C]oleic acid labeled E. coli Y1090 incubated alone. See FIG. 21.

Example IV - Searching for human cDNA and Genomic DNA Sequences homologous to $RPLA_2$-8

Two primers, clo8-4 and clo8-5, synthesized according the published human h8 presumptive exon II sequence, Seilhamer, J.J.: J. of Cellular

Biochemistry, 39:327-329 (1989), are used in a PCR amplification screen of human child brain, adult brain, liver, heart, and various white cell cDNA libraries. No PCR amplification is obtained from any of them. Two overlapping human genomic DNA clones, clone 8 and walk 9, containing 10 kb of DNA 5' of h8 exon II and 16 kb of DNA 3' of h8 exon II, respectively, are then analyzed. Southern blot analysis using the PCR fragment containing the RPLA2-8 open reading frame DNA sequence as a probe identified two EcoRI fragments, one in clone 8 and one in walk 9. These two fragments are subcloned into pUC19 and sequenced. DNA sequence homology between these sequences and the RPLA2-8 cDNA indicated that one fragment contains a region homologous to RPLA2-8 exons I and II, and that the other fragment contains a region homologous to RPLA2-8 exon IV. See FIG. 16. In order to search for exon III of a human RPLA2-8 homologue, the entire region between exon II and exon IV is sequenced. No region homologous to RPLA2-8 coding exon III is found by computer analysis of this sequence. To determine if the HPLA2-8 sequence is transcribed, two primers, one in coding exon II and one in exon IV, are used to do RT-PCR of human brain and lymphoblast total RNA. No PCR amplification signal is obtained.

Example V - Phospholipase A_2 assay using aut - claved labeled bacterium as a substrate

Autoclaved [1-^{14}C]oleic acid-labeled E.coli 1-^{14}C 109 is prepared according to the methods described by Elsbach: P. et al.: Methods in Enzymology, 197:24-31 (1991) for use as the PLA_2 substrate. Commercial porcine pancreatic PLA_2 (Sigma) is used for the standand assay. Simply, the serialy diluted PLA_2 solutions are incubated for 15 minutes at 37°C with E.coli substrate (a mix of $2.5x10^8$ labeled and unlabled bacteria to provide 10,000 cpm) in a total volume of 250 ul (40mM Tris/HCl, pH 7.8, 10mM Ca^{+2}). The reaction is stopped by the addition of 250 ul ice cold 0.5% (W/V) fatty acid-poor BSA (USB). After incubatation on ice for 5 minutes, the samples are centrifuged at 10,000 x g for 2 minutes, and 250 ul of the supernatant containing released [1^{-14}C]oleic acid is counted in a scintillation counter.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Tischfield, Jay A.
Seilhamer, Jeffrey J.

(ii) TITLE OF INVENTION: Mammalian Phospholipase A2 Nucleotide Sequences and Low Molecular Weight Amino Acid Sequences Encoded Thereby, Antisense Sequences and Nucleotide Sequences Having Internal Ribosome Binding Sites

(iii) NUMBER OF SEQUENCES: 44

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ruden, Barnett, McClosky, Smith, Schuster & Russell PA
(B) STREET: 200 East Broward Boulevard
(C) CITY: Fort Lauderdale
(D) STATE: FL
(E) COUNTRY: USA
(F) ZIP: 33301

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/097,354
(B) FILING DATE: 26-JUL-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Manso, Peter J.
(B) REGISTRATION NUMBER: 32,264
(C) REFERENCE/DOCKET NUMBER: IN21044-5

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 305-527-2498
(B) TELEFAX: 305-764-4996

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Lys Leu Leu Val Leu Ala Val Leu Leu Thr Val Ala Ala Ala

1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Ser Gly Ile Ser Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Val Trp Gln Phe
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Lys Thr Leu Leu Leu Ala Val Ile Met Ile Phe Gly Leu Leu Gln
1 5 10 15

Ala His Gly

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asn Leu Val Asn Phe
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Leu Leu Val Ser Ser Gly Met Lys Gly Ile Ala Val Phe Leu
1 5 10 15

Val Phe Ile Phe Cys
20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Trp Thr Thr Ser Thr Leu Ser
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ser Phe Trp Gln Phe
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Lys Gly Leu Leu Pro Leu Ala Trp Phe Leu Ala Cys Ser Val Pro
1 5 10 15

Ala Val Gln Gly
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Leu Leu Asp Leu
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Lys Arg Leu Leu Thr Leu Ala Trp Ph Leu Ala Cys Ser Val Pro
1 5 10 15

Ala Val Pro Gly
20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gly Leu Leu Glu Leu
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTGGCTTGGT TCCTGGCTTG TA 22

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAAGGAGGC TTGCTGGACC TA 22

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATCGGTGCCA TCCTTGGGGG TT 22

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAGAGGATG TTGGGAAAGT AT 22

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GAATTCGCTA GCCAGAGATG AAAGGCCTCC TCCCACTGGC TTGG 44

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCGCTCTCG AGGCCCTAGG AGCAGAGGAT GTTGGGAAA 39

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGCCACGCGT CGACTAGTAC T 21

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCCACGCGT CGACTAGTAC 20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4325 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 722..1195

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GAATTCCGCC TCCACCTCTC AAATGCTGGG ATTGCAGGAT GTCCCCCCAC CCCTGCTCCC 60

TTGTGTCCTT GCTTCCTGCT GCCGGAATGT ATCACTTAAT TGCCAGGTAC CCATGGTCTG 120

ATTCCAGGAT AGAAGGGCGG GCGAGGGGGT TGGAGGAGAG GCCTCTATTA TTTCCGCGGT 180

CTGGCAGGCC TGGAAGCAAA GCTTCAAGTG CAGAAGGAGG AGTGTCGGGG AATGGCAGAA 240

AAGGCTGGAA CAGCAATGCA GACCTAGGTA AAGGGCACAG AGCTGAGGGA AGCTCCTGGG 300

AGGCTCCCTG CAGCTCCTGC CTCTGCACAT GACCCGGACT CCTTTTCTCT CTTTGGATCT 360

GCGTCCAGGG ACTGGCTTGT ACACACCCCT CCCAGGAGAC CCCTTGGCAG CTGCACACTC 420

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AGGCTCCATC CAAGTTGGCT CTGCCCCTGG GGAAGGCTGC TCAAAAGGCC TGGCTCCCAG      480

TTTCTGGGGA CCCACAGAGA GCCTCTCACC TCGCAGCTCA GCTCCATCCG CCTCCTGTGC      540

CTGGCTGCGA CCAGCTGGGT CTAACTATAG ACAGTCAGCA ACTTCAGCCA CTTCACCGAG      600

TTTCCCAACA GCTTTGAGAT TTGGAAGCCG GAAGCCTGAT CGCCTTCTCA GAAGCTACGG      660

TCCACTACCT CAGCCATTCT GTTGGAGCTG AACTGGCAGA TGAAGGTGAG ACCCAGGCAC      720

C ATG GAC CTC CTG GTC TCC TCA GGA ATG AAG GGC ATC GCT GTC TTC           766
  Met Asp Leu Leu Val Ser Ser Gly Met Lys Gly Ile Ala Val Phe
    1               5                  10                  15

CTT GTC TTT ATC TTC TGC TGG ACA ACC TCC ACC CTC AGC AGC TTC TGG        814
Leu Val Phe Ile Phe Cys Trp Thr Thr Ser Thr Leu Ser Ser Phe Trp
                20                  25                  30

CAG TTC CAG AGG ATG GTC AAA CAC ATC ACG GGG CGC AGC GCC TTC TTC        862
Gln Phe Gln Arg Met Val Lys His Ile Thr Gly Arg Ser Ala Phe Phe
            35                  40                  45

TCC TAT TAC GGA TAT GGC TGC TAC TGT GGG CTT GGG GGC CGA GGG ATC        910
Ser Tyr Tyr Gly Tyr Gly Cys Tyr Cys Gly Leu Gly Gly Arg Gly Ile
        50                  55                  60

CCT GTG GAC GCC ACA GAC AGG TGC TGC TGG GCT CAT GAC TGT TGC TAC        958
Pro Val Asp Ala Thr Asp Arg Cys Cys Trp Ala His Asp Cys Cys Tyr
    65                  70                  75

CAC AAG CTT AAG GAA TAT GGC TGC CAG CCC ATC TTG AAT GCC TAT CAG       1006
His Lys Leu Lys Glu Tyr Gly Cys Gln Pro Ile Leu Asn Ala Tyr Gln
80                  85                  90                  95

TTT GCC ATT GTC AAC GGG ACC GTG ACC TGT GGA TGC ACC ATG GGT GGC       1054
Phe Ala Ile Val Asn Gly Thr Val Thr Cys Gly Cys Thr Met Gly Gly
                100                 105                 110

GGC TGC TTG TGC GGG CAG AAA GCC TGT GAG TGT GAC AAA CTG TCT GTG       1102
Gly Cys Leu Cys Gly Gln Lys Ala Cys Glu Cys Asp Lys Leu Ser Val
            115                 120                 125

TAC TGC TTC AAG GAG AAC CTG GCC ACC TAC GAG AAA ACT TTC AAG CAG       1150
Tyr Cys Phe Lys Glu Asn Leu Ala Thr Tyr Glu Lys Thr Phe Lys Gln
        130                 135                 140

CTC TTC CCC ACC AGG CCC CAG TGT GGC AGG GAC AAA CTC CAT TGC           1195
Leu Phe Pro Thr Arg Pro Gln Cys Gly Arg Asp Lys Leu His Cys
    145                 150                 155

TAGGCCTTCC CCTCCAAGAG TCCCCAGGCT CCTGCAGCTC AGCCTTGCTG TCTAGGGAGT      1255

GTCTTCTCAG GCATTAGGGG ACCGGAGGTG GAGAATTCCT GCCCTGGAAT CAGACCATGG      1315

GTACCTGGCA ATTAAGTGAT ACATTCCGGC AGCAGGAAGC AAGGACACAA GGGAGCAGGG      1375

GTGGGGGGAC ATCCTGCAAT CCCAGCATTT GAGAGGTGGA GGCAAGAGGT GGGGGGTAGC      1435

CTCCACTATA CGGTAAGTTC AAGGCTAACC TGAGCTACCT GAGACCTTGC CTTGAAAAAA      1495
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CTTTTTTAAA AAACGTTTAA AGGAAAAGAA AACAGAAAGA CACGGGGACT GGGCTGAAAG 1555

GTACTCTCAA ACCGATTTCC CAGGAAGAGC GGAGAGCCCC AGGTTCAGCT CCAGCCTGAA 1615

CTCCCCCATA CCCTCAGTCC TGGTCAGGAT GTGTGTCTGA CTGGGGAACC AAGTCCTCCA 1675

CCCGGGTGGA GCTTAGCTGG GAACTACGCA GGTGTCCTAG AAAATACAGT CCTAAGAGCC 1735

TCACCCGGAG TCTCATCCCC ATTTGCTCCA GGACTGACCT CTGTAAATCT TCCAGCAGGA 1795

AGCAGGCTGT ACCCATCTCA GGAGGTGGGG TGCTGTTAGA ACAATGGTGT GCACCAGTGA 1855

CACAAAGATG TCATGGTTAA GATGGCATCA AGAAGTGGAA AGGACATTCG GAACAGTGGG 1915

TCCAAGGCAC CCAAAGTCCT CACCCCAATT TAGAAGCCGT TGGTCCTGTA AGACTTAAAT 1975

CTACTAAACA AGGAAGGTCT AACTGGGCTG GAATCTGAAG TTCATGGTGC CAGGCTGGGG 2035

CGGTGGGTGG GGACGTGGCC GTGGCCATGA CCATGATTGC CTCTCTGCAT GGTGACACTT 2095

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GAATCTGAGA CTCTCCTCAC TAATGTAGCA AAAATGGAGG TCTAAAGTGC AGGCTTCAGC 2215

CTCTGAGGTC CAGGGCAGGA GGAAGCTGGG GCTCAGCCTC CTGGAGGATG AGAGCTTGCC 2275

GGGTGAGCAT CAGCGACAGC AGACCCTTGG GCTCAGAGAG TCCGCAAGCC TGGGAGAGCC 2335

TGGCCGAGCC CTGACTGCAG CACACAGAGC CGTGAGCCTC ATACAAGAAG CCACATTTTG 2395

GGGAAGCTTC AGGGTGGCTG ATTCCACAGC TGTTGGGTTC AGAACGGAAG CCGGGAGCAC 2455

TCACTTCAGA TATGGAAGCT TTGTTTTACG AGCGCTTAGC ACCAGTTCAG GATCTGAACT 2515

TCGTCCTGAC CGGAGAGTCC GTACCACATT TTTATAGGAT GGGAACACAG AGCGAGGGGC 2575

GTGGAGTAAG CTGTTGAACG ACCGATCATA TTTTGACCTA AGAGGTTAAG TAAGGACGTT 2635

AACATGGGTG ACTGGGCATT AGTCAGGTCA CCTGGTTTTG GGGTCTTTGA ATCAGCTTTC 2695

GTGGCCAGGT CCCTTCCTGG ACTTTGGCTC GGAATTTAGA ACGATAAGGG AACGAAGAGG 2755

TGGGCAAGCT TCGGGCAGTC AGTAAGAGGC AGCACATTCA TGACCTGTGT GCCTTGTTTA 2815

GATAATGGGA TAAGAGTATC TCCTCTCTTA CACCCCTTAC TGGTTAACAG ACAAACACGA 2875

GACATCTGAA GAAGCAGGAC AGGAGTTAGG TTCTGGGGCA CAGGAACATG AACTCGGTTT 2935

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TAACAAGTTA GTTGATGGCG CTGGTCCTTA GTCACCGATA CTGTCAGGCT CTCAGCTCTT 3055

GGGCCAGACT TGGCGGCCAT GGGAGTGTGG TCACTTGCCC CGTCCCCTTC TTCCAGGAGG 3115

TACTGGGGAA AATGGTTGGA TTTGTGGAGT TGTAGGGAAC ACTCATGGCT CCCTTCACTT 3175

AGTAGGTCAG CTAACATATG TGTATCGAGC CCATACCGTG TGCCATGTGC AGTGCTGAGC 3235

AGCAGGGAGT CAGAGATTTA AAGACACACA CACAGACTTC AAGTCTGAGA ATTTTGAATC 3295

CCAGGGAGAA CCGCTGAGAG CCATGGCGCT TCTACCAATG CCAGAGGCTA ACACCCGGAC 3355

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GAGCAGTACC TAGTTCAAAT CACAGAGTCG TCTTTCTTCC TCCACCCTAC CCAGGTACAG 3475

CAAGTAGACA CGGGTGGGGG CAGGGCAGGG ATGCAGGAAC ATTAGGGCAC ACCGATGTGG 3535

CTAGGCTAAG CTAGAGCATG TTACCTTCTC AGGGGTCCTG TCATGTCAGA GACTGGTTCC 3595

AACCTGGAAA GATGTCTGAG TGACAGCTGT GGTAGAAGAA GAGAGGCCAG GGTGATATCA 3655

GCATGAAGGG CTGGATTGCT ATGTGAGATC CAGATCTCTT CTGCCACTGG GGTCAGCTTC 3715

TACACTGGAA ATAGATGGGC TGCGTTATGG AGGGTGGTGT GAGTCCCTGT CTGCGTTGTG 3775

CCGGGAATCA GAGCAGAGTG TTAGCGCTGT AAAAGGACAT GCTGGTGTTT GCAGGAAATC 3835

ATCGATTTCT TGGAAGGGCA GCCATTCATC TACACCAGGG ATTGACTTTA TGCCAGGCTT 3895

GTGATGAGGG TAGAAAAGTA GAAATTCTGT CCGCTGCAAG GAGCAGTCAG AGGACACAAG 3955

GACCAAATAG CTTGGGAGTT GCGGAAGTAG GTGTCTGCTG AGGGAGCAGT GACCACTGGG 4015

GGAAAGGCTC CTTCAAGGAA TTCAGGGACA GGGGTGAGGG CTGACATCTT GCCTGAGACC 4075

CTAAAGAAGA GAAGGAGTTG AGAGGGCTGA GTATGCTGTG TGGAGCCCCA CCCCCACCCC 4135

CACCCCCACC CCCACCCCAG GTATATGGAT GGAGGATAAT GCGGGGGTCG GGTTCCTCTC 4195

AAATCCATCA TCCCACCTTC GAGCTGCTGG CACGGCCTTG CCAGCACAGC CCGATTCTGT 4255

GTTGACAAAA TACTCGAACG AAATGATTAC ATGCAAATAA AATGCAAGAG GAAAAATCTA 4315

AACGGAATTC 4325

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 158 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Asp Leu Leu Val Ser Ser Gly Met Lys Gly Ile Ala Val Phe Leu
1 5 10 15

Val Phe Ile Phe Cys Trp Thr Thr Ser Thr Leu Ser Ser Phe Trp Gln
20 25 30

Phe Gln Arg Met Val Lys His Ile Thr Gly Arg Ser Ala Phe Phe Ser
35 40 45

Tyr Tyr Gly Tyr Gly Cys Tyr Cys Gly Leu Gly Gly Arg Gly Ile Pro
50 55 60

Val Asp Ala Thr Asp Arg Cys Cys Trp Ala His Asp Cys Cys Tyr His
65 70 75 80

Lys Leu Lys Glu Tyr Gly Cys Gln Pro Ile Leu Asn Ala Tyr Gln Phe
85 90 95

Ala Ile Val Asn Gly Thr Val Thr Cys Gly Cys Thr Met Gly Gly Gly
100 105 110

Cys Leu Cys Gly Gln Lys Ala Cys Glu Cys Asp Lys Leu Ser Val Tyr
115 120 125

Cys Phe Lys Glu Asn Leu Ala Thr Tyr Glu Lys Thr Phe Lys Gln Leu
130 135 140

Phe Pro Thr Arg Pro Gln Cys Gly Arg Asp Lys Leu His Cys
145 150 155

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ACCTCAGACC CCCTGGTCTC CTCAGGAATG AAGGTCATTG CCATCCTCAC CCTCCTCCTC 60

TTCTGCT 67

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ACCATGGACC TCCTGGTCTC CTCAGGAATG AAGGGCATCG CTGTCTTCCT TGTCTTTATC 60

TTCTGCT 67

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TGGTGGCAGC CCCCACCCAC AGCAGTTTCT GGCAGTTTCA GAGGAGGGTC AAACACATCA 60

CGGGGCGAAG TGCCTTCTTC TCATATTACG GATATGGCTG CTACTGTGGG CTTGGGGATA 120

AAGGGATCCC CGTGGATGAC ACTGACAGGT G 151

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CAGGGACAAC CTCCACCCTC AGCAGCTTCT GGCAGTTCCA GAGGATGGTC AAACACATCA 60

CGGGGCGCAG CGCCTTCTTC TCCTATTACG GATATGGCTG CTACTGTGGG CTTGGGGGCC 120

GAGGGATCCC TGTGGACGCC ACAGACAGGT G 151

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TAGGTGGATG CACCCTTGGT CCTGGTGCCA GCTGCCACTG CAGGCTGAAG GCCTGTGAGT 60

GTGACAAGCA ATCCGTGCAC TGCTTCAAAG AGAGCCTGCC CACCTATGAG AAAAACTTCA 120

AGCAGTTCTC CAGCCGGCCC AGGTGTGGCA GACATAAGCC CTGGTGCTAG 170

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 167 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
CAGGTGGATG CACCATGGGT GGCGGCTGCT TGTGCGGGCA GAAAGCCTGT GAGTGTGACA      60

AACTGTCTGT GTACTGCTTC AAGGAGAACC TGGCCACCTA CGAGAAAACT TTCAAGCAGC     120

TCTTCCCCAC CAGGCCCCAG TGTGGCAGGG ACAAACTCCA TTGCTAG                   167
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1828 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 233..643

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
GAATTCCGGT GGATGGAGGG GGCTGAGCAG GATGTTGACT GGCTATCGTT CATTGAGCAC      60

TCTCACGATC AGCATCACGC ACGGAATCCA TCCTTCCTGT GTTGCAGCTT GTAGACCCTG     120

ATGCTTGGGC TGCCAGCATA AACGTGGGGA TCCAGACTCT GTCTACCGAG GCTGCCCATA     180

GGGACAGGCC CTGGGAAGAG GAGCTGAGAC CAGGCTAAAA AGAACCCAAG AA ATG         235
                                                            Met
                                                             1

AAG CGC CTC CTC ACG CTG GCT TGG TTC CTG GCT TGC AGT GTG CCT GCA       283
Lys Arg Leu Leu Thr Leu Ala Trp Phe Leu Ala Cys Ser Val Pro Ala
          5                  10                  15

GTC CCA GGG GGC TTG CTA GAA CTG AAG TCC ATG ATT GAG AAG GTG ACT       331
Val Pro Gly Gly Leu Leu Glu Leu Lys Ser Met Ile Glu Lys Val Thr
      20                  25                  30

GGG AAG AAT GCC GTA AAG AAC TAT GGC TTC TAC GGC TGC TAC TGT GGC       379
Gly Lys Asn Ala Val Lys Asn Tyr Gly Phe Tyr Gly Cys Tyr Cys Gly
  35                  40                  45

TGG GGC GGC CAC GGG ACC CCT AAG GAT GGC ACT GAT TGG TGC TGT CGG       427
Trp Gly Gly His Gly Thr Pro Lys Asp Gly Thr Asp Trp Cys Cys Arg
 50                  55                  60                  65

ATG CAC GAC CGT TGT TAT GGG CTA CTG GAG GAG AAA CAC TGT GCC ATC       475
```

```
Met His Asp Arg Cys Tyr Gly Leu Leu Glu Glu Lys His Cys Ala Ile
                  70                  75                  80

CGG ACC CAG TCC TAT GAC TAC AGA TTC ACA CAG GAC TTA GTC ATC TGC      523
Arg Thr Gln Ser Tyr Asp Tyr Arg Phe Thr Gln Asp Leu Val Ile Cys
              85                  90                  95

GAA CAC GAC TCC TTC TGT CCA GTG AGG CTT TGT GCT TGT GAC CGG AAG      571
Glu His Asp Ser Phe Cys Pro Val Arg Leu Cys Ala Cys Asp Arg Lys
         100                 105                 110

CTG GTC TAC TGC CTG AGG AGA AAC CTC TGG AGT TAC AAC CGT CTT TAC      619
Leu Val Tyr Cys Leu Arg Arg Asn Leu Trp Ser Tyr Asn Arg Leu Tyr
     115                 120                 125

CAG TAT TAC CCC AAC TTC CTC TGC TAATGTCCTC TGTGGGCTCT CGCCGGGAGT      673
Gln Tyr Tyr Pro Asn Phe Leu Cys
130                 135

GCCTCCCACA GTGGCGGCCC CCCTCGGCTG TATTCCTGAT CCGTCCACCC AAGGTCTTGG     733

ATCTGCCTTC CTCTGTGTAC CACTGGGCTG GACAGAGCCC AGGGTTACAC CCTACCCTCC     793

AGAATCCTAG AGAGGGACTC TGATGTAGAG TCTGCGGACT CTGGATAGCT GAGCCTGCAC     853

TTGCAGAATT TGGCGCTGGG CCCCGGAGCT CCCTCAGCTC CAGGCCAGTG TCGTGTTGAC     913

TTTCCTTTCA ATTTCTGGAA CCCAACTGCC ATTACCACCC TCCAGAGACC TCTTACTAGA     973

GGAGAAGCCA AATTAACTCT ATAAATCTGC CATGTAGCTA TTAAATAAAA CCCATTCACG    1033

AGGCGAGAAG AACACCACCC CAGCACTCCC TCTGACAGGG CTGGGGTAGG AGTGCCAATG    1093

CTTCTCTAAC CCCTGAGGCA TCTGTGCACC CTCTAGGATG GAGGTCAGGA AACAGGTGGG    1153

GGCCTTACAT GCCTTTCATG GTTTGTCTTG AGTTTATTTT CTTAAACCTT AGGGTCTTTC    1213

AAGCCAGACC TGGAGCTCAA GATTCTTCTG GAGGAAGGTG AGACACAGCC CTATGCCACC    1273

TTGAGCTCCA GGCTAGAAAG GGACAGCCCC TAGCCCTGGC TTCTGCAACT GTGTGGTCTT    1333

GAACCTCCGT ATAGTCCGAA TCCCTCTGGC TCTCCTCAAA ATATAAAACA AGCCTCCTTC    1393

CAATAGCATA TTGGTGCACA CCCCTAATCC CATCACCTGG GAGGAGGAGG CGGCAGGAGC    1453

ATCAGGAGTT CAAGGCCAGC TCCTGCCCCC TAGCAGGGAT GGTAGGCTGC ATGAGAGTGT    1513

GTCTCAGAAA GAACCACCTG GTGCGGGTAC AGGGATGCTG GGATTCTGAG ATGTCACTCA    1573

GTGCGGGAAA AGATTCAAGG AGGGGAACAG ATCAATGGCA GAATGACTGT CTGTGCCGAG    1633

TTAAGGGCAC TGAAAATCTC AGCTCATCTA TCGCTTTATA GAAGATAGAG CTTTGGGAGG    1693

AAGCAAGGCA CTCTACAGTA AAGGAGTGGC CTTTCCAAGG AAGGGTCTAG GCTCCTTCTT    1753

CTCCAGAACA TGCACAGGAC ATAGGAGATC CATTATTTAG AGACCTTTCG TGTTCGAACG    1813

TTTTCTCCGG AATTC                                                     1828
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 137 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Met Lys Arg Leu Leu Thr Leu Ala Trp Phe Leu Ala Cys Ser Val Pro
1 5 10 15

Ala Val Pro Gly Gly Leu Leu Glu Leu Lys Ser Met Ile Glu Lys Val
20 25 30

Thr Gly Lys Asn Ala Val Lys Asn Tyr Gly Phe Tyr Gly Cys Tyr Cys
35 40 45

Gly Trp Gly Gly His Gly Thr Pro Lys Asp Gly Thr Asp Trp Cys Cys
50 55 60

Arg Met His Asp Arg Cys Tyr Gly Leu Leu Glu Glu Lys His Cys Ala
65 70 75 80

Ile Arg Thr Gln Ser Tyr Asp Tyr Arg Phe Thr Gln Asp Leu Val Ile
85 90 95

Cys Glu His Asp Ser Phe Cys Pro Val Arg Leu Cys Ala Cys Asp Arg
100 105 110

Lys Leu Val Tyr Cys Leu Arg Arg Asn Leu Trp Ser Tyr Asn Arg Leu
115 120 125

Tyr Gln Tyr Tyr Pro Asn Phe Leu Cys
130 135

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1014 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 131..544

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGATACCAAT GTTCCGACTG GAGACGGGGA GCCCGCGAGA CCCGGGTCTC CAGGGTCTGC 60

CCAAGGAAGT TGCTCATGGG AGCAGACCCC TAGAGCAGGA TTTGAGGCCA GGCCAAAGAG 120

```
AACCCCAGAG ATG AAA GGC CTC CTC CCA CTG GCT TGG TTC CTG GCT TGT          169
           Met Lys Gly Leu Leu Pro Leu Ala Trp Phe Leu Ala Cys
             1               5                  10

AGT GTG CCT GCT GTG CAA GGA GGC TTG CTG GAC CTA AAA TCA ATG ATC          217
Ser Val Pro Ala Val Gln Gly Gly Leu Leu Asp Leu Lys Ser Met Ile
     15                  20                  25

GAG AAG GTG ACA GGG AAG AAC GCC CTG ACA AAC TAC GGC TTC TAC GGC          265
Glu Lys Val Thr Gly Lys Asn Ala Leu Thr Asn Tyr Gly Phe Tyr Gly
 30                  35                  40                  45

TGT TAC TGC GGC TGG GGC GGC CGA GGA ACC CCC AAG GAT GGC ACC GAT          313
Cys Tyr Cys Gly Trp Gly Gly Arg Gly Thr Pro Lys Asp Gly Thr Asp
                 50                  55                  60

TGG TGC TGT TGG GCG CAT GAC CAC TGC TAT GGG CGG CTG GAG GAG AAG          361
Trp Cys Cys Trp Ala His Asp His Cys Tyr Gly Arg Leu Glu Glu Lys
           65                  70                  75

GGC TGC AAC ATT CGC ACA CAG TCC TAC AAA TAC AGA TTC GCG TGG GGC          409
Gly Cys Asn Ile Arg Thr Gln Ser Tyr Lys Tyr Arg Phe Ala Trp Gly
       80                  85                  90

GTG GTC ACC TGC GAG CCC GGG CCC TTC TGC CAT GTC AAC CTC TGT GCC          457
Val Val Thr Cys Glu Pro Gly Pro Phe Cys His Val Asn Leu Cys Ala
     95                  100                 105

TGT GAC CGG AAG CTC GTC TAC TGC CTC AAG AGA AAC CTA CGG AGC TAC          505
Cys Asp Arg Lys Leu Val Tyr Cys Leu Lys Arg Asn Leu Arg Ser Tyr
110                 115                 120                 125

AAC CCA CAG TAC CAA TAC TTT CCC AAC ATC CTC TGC TCC TAGGCCTCCC           554
Asn Pro Gln Tyr Gln Tyr Phe Pro Asn Ile Leu Cys Ser
                130                 135

CAGCGAGCTC CTCCCAGACC AAGACTTTTG TTCTGTTTTT CTACAACACA GAGTACTGAC       614

TCTGCCTGGT TCCTGAGAGA GGCTCCTAAG TCACAGACCT CAGTCTTTCT CGAAGCTTGG       674

CGGACCCCCA GGGCCACACT GTACCCTCCA GCGAGTCCCA GGGGAGTGAC TCTGGTCATA       734

GGACTTGGTA GGGTCCCAGG GTCCCTAGGC CTCCACTTCT GAGGGCAGCC CCTCTGGTGC       794

CAAGAGCTCT CCTCCAACTC AGGGTTGGCT GTGTCTCTTT TCTTCTCTGA AGACAGCGTC       854

CTGGCTCCAG TTGGAACACT TTCCTGAGAT GCACTTACTT CTCAGCTTCT GCGATCAGAT       914

TATCATCACC ACCACCCTCC AGAGAATTTT ACGCAAGAAG AGCCAAATTG ACTCTCTAAA       974

TCTGGTGTAT GGGTATTAAA TAAAATTCAT TCTCAAGGCT                            1014
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 138 amin acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Lys Gly Leu Leu Pro Leu Ala Trp Phe Leu Ala Cys Ser Val Pro
 1               5                  10                  15

Ala Val Gln Gly Gly Leu Leu Asp Leu Lys Ser Met Ile Glu Lys Val
            20                  25                  30

Thr Gly Lys Asn Ala Leu Thr Asn Tyr Gly Phe Tyr Gly Cys Tyr Cys
        35                  40                  45

Gly Trp Gly Gly Arg Gly Thr Pro Lys Asp Gly Thr Asp Trp Cys Cys
    50                  55                  60

Trp Ala His Asp His Cys Tyr Gly Arg Leu Glu Glu Lys Gly Cys Asn
65                  70                  75                  80

Ile Arg Thr Gln Ser Tyr Lys Tyr Arg Phe Ala Trp Gly Val Val Thr
                85                  90                  95

Cys Glu Pro Gly Pro Phe Cys His Val Asn Leu Cys Ala Cys Asp Arg
            100                 105                 110

Lys Leu Val Tyr Cys Leu Lys Arg Asn Leu Arg Ser Tyr Asn Pro Gln
        115                 120                 125

Tyr Gln Tyr Phe Pro Asn Ile Leu Cys Ser
    130                 135
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15328 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
AAGCTTTGTG GGATTTCTAT TATGAACAAC ATAGGTGCCT TTCCAACTCG GGAACAGAGG      60

AAATATGGAC TCCTCAAAAG AAAAAAAGAA GAGATGAAGG GATGATGTTG CCAAAGAAAG     120

AAATTTGGAA AAAAAAAAAC CAAACCAACA TTTGCACTTT CAAAACCATG GAACCCTTCT     180

TATTTTTATA TGTTCAGATC TAAATGCCAG AAAGGTTACC ACATTCAAAG GGAATGAGAT     240

TTGAAAATGA TTTCTTTGAG TCCTCTGCTG AGGTCTTTCC AAGGCACTAC AATTAGGGCT     300

TTGCACCCAA ATACCCTTGC CTCATTTTGG TCATTTTTGT CCTGGAACAG AGGTTCAGCT     360

GGGAGACCCC TCACACACAG GTGAAGGCGT GGCTGTAGAA CCTCAGACCC CCTGGTCTCC     420

TCAGGAATGA AGGTCATTGC CATCCTCACC CTCCTCCTCT TCTGCTGTAA GTAGAGAGCG     480
```

TTGGTGGGTC AGCACCAAGC TTCTGTCTTC CTGTTTATGT CAGTGGGAGG GGGGACTCTC 540
CAGGTGGCAC CAGGTGAGGG AAGTCACAAG TCCCGCAGAA AAGAATCAGG AAAGGAACGG 600
GCTCCCACCA ACGTCCTCTT GCTTCTGTTT CTGCTATAAA ATGGGCTGAT CCCAGTGTTG 660
GGATCTTATA AAGTGTCTAG GAAATCAGAG GTTGCCAACC ATTTGCTAGA AAGGGAGTTT 720
GAGTAGTATT TTACCCCCCC TCACCCTCAA GAGTCTTTTT ACTTTGGATG CTAGTAGCCT 780
TTTATTTAGG CATTGGATCA GAACAAAAAT GCAGGACATA TATCCAGCCT AATTTAACCA 840
ATGGATTAAA TGGCCTTATC AGGAAAAGAC CATTTTATGG TGACTTATGG GATAATTGGT 900
AGTTATAAGT CATTGCTGCC GGGAGATCCG ATTGCTTACC TCTGCAAAGT GAAGAAAGAC 960
CTACTGGGAA ACAGTTTGGG GTCTACTGGA GACTGATAGA CTCTTTTGCT GGATTCGTTG 1020
AGTGGAGGTT TCTCCAGATC CATTTTCCTG TCTCTTTCAA TTGAGTCACA ATAACTTTTG 1080
AGTCCCTAAG TCAAAGATGT CAAAAACAGA CTTCCTTTCC CCACAGTGAG TGGTGGAATT 1140
TACACTTTGC AAGGTGATAG TGCAGGAGGA TACCTGTACG CAGGGATGAC CGCCTCTGCA 1200
GCCCTCAGTG CGGCTCCAGG ACTGCTTGGG CACCAGTGAC CGCCCCATGG GTTTCTTCCG 1260
CCACACCCCC GTTTAGACTG AACACGATAG GTAGATCGAA GGCCACCTGA GAAAACTCCC 1320
CCAAAACTCT ATTTCTGTTT CTCTTCTTCA AAGTTCATGT CTTTGTTGTA TTTTTATTGC 1380
AAATTTACTA CATGCTTATA GTTAAAAAGT AAAATAAATG AGTATATAGC AACAAGGTAA 1440
AGCTCCTCCT CATCCTCCCC AGACCCCAGT TTTTTCCCTA CATCCAGATG TGACCACTCT 1500
TAAGAGTTTG ATATACATCC TCTATACAGC GTTTACCACA CACACATTCA AAACACCATA 1560
ATAGGAAGGG AACACATGCT GGGCCGGGCG CGGTTGTTCA TGACTATAAT CCCAGCACTT 1620
TGGGAGGCCG AGGCGGGCGG ATCACCTGAG GTCAGGAGTT CGAGACCAGC CTGGCCAGCT 1680
GGCAACATGG TGAAACCCGT CTCTATTAAA AATACAAAAA ATTAGTCAAG CATGGCAGTT 1740
GGGCACCTGT AATCCCAGCT ACTCAGGAGG CTGAGGCAGG AGAATTGCCT GAACCCGGGA 1800
GGCGGAGGTT GCAGTGAGCC GAGATCACAC CATTGCACTC CAGCCTGGGT AACAACAGCG 1860
AAACTCCGTC TCAAAAAAAA AAAAAAAAGA AGGAAAGGGA CACACGCTTA TTATGAAAGA 1920
CATGAGACAG CGGAGACGTG TATAAATGAT GTTGCCTGTT TTCTTTCTCT CTCTTCATCC 1980
ATGCTAGAGA TAGTGCTATC AAATGTAGTT ATTTTTGAGA CACATATTTC GTATTATCCC 2040
TGTCGTGACA TGTGGGTGGT TTCCAATTTT TTGATATCAC AGATAATGCT TCAGGAAACC 2100
ATTTTGTGTA TCGATTTGTG CCCACTCTCA TAAGCATCTT GTAGAAGCAA AAACAGCTGA 2160
GTTCATGTGT ACTTGTCATT TAAAAAAATA ATAATTGAGG ATACCTTTCC TGCCTCTTAA 2220
GTATTTTGTT TCTCCTGTGA GATAGTAAAG GCCTGATGAC ATCTGGAGGG ACTGGCGTTT 2280

CTGGCTTTGA ACTTTTGCCA TTCATGTTGC ATCAGACCCG AGGGTGTTCT GCCTAGAACT 2340

GTGGTTTCTT GCTTTGAGGG GGAAGACTAT GGTTGATGGG AAAGCCTTGT TCTGAACCTC 2400

ATGGAAACTG GGTATTCATC TGGGTTAGCA AAAAACTAGC TGTGTTACAG GGGCAAATCT 2460

GAACCTATTT TATTCCCCAG GAAAGAGGCT GGTGATTCCA GCCATGCCCC TTGCACTTCG 2520

CTTTGGGGAT CTGGTGATAT TTCGAATGCT CAGCACTCTA GTAAGGGGAG GGGACATCAA 2580

GGCAGCATCA TGCTCATTGC AACTTCCTTC TTCCTTTTTT TCTCATCGGT GGTGGCAGCC 2640

CCCACCCACA GCAGTTTCTG GCAGTTTCAG AGGAGGGTCA AACACATCAC GGGGCGAAGT 2700

GCCTTCTTCT CATATTACGG ATATGGCTGC TACTGTGGGC TTGGGGATAA AGGGATCCCC 2760

GTGGATGACA CTGACAGGTG GGTGCAGAGG CTCTAAGGCC ACTTATCATT TGTTTTGCAT 2820

TAAAGTTCAT GCTCAAAGCC AGAGAGAGGG TCTTAGGATT CTTGCCTGGC AAATAACAGA 2880

AAACAACTCA GGCTAATGGA AGGAAGAACT GAACGGGATT TGGAGGATGG GTCTTGAGAA 2940

ACCCAGGGTC GGGGCCAGCT TCTTGAGTGT GTGACCTGTG AAGTTTCACA GGGCCCAACA 3000

CTCATAAGGG TCAGGGCCAG CTTCTTGAGC GTGTGATCTG TAAAGTTTCA CAGGGCCTGG 3060

CACTCATAAC CCCCTAAACA TGGTTTACTG CTCTGCTGCC ACATCTTGAA ATTCTTAATA 3120

AAGGGCCTCA TGTTTTCATT TTGCTTTACT CTCTGCAATT ATGCCGTTGG TCCTGCCCAG 3180

AGCTCTAGAA GCTGTTTCAT CCTCATAGTA AAAGTGCTCT GCTTTCAGCT CTCCAGCTTT 3240

TAGCACTATA CCCACAGCAC AACTGACTCA CTAGTCCTAA TTCCATATTC TGGAGAGGGC 3300

TCCAAAGTGG CCCACTTTGG AGAAGTTGTC CATCTGGGTG AGGTTGCATG GCACAAACCT 3360

GGCTTCAGGC CTACTCCAAA GGATGGGGGT GGGGGAGTGT GAGTTCCTAG AAAAAGTAGA 3420

GGTGGGTGTC ATCTGGTGAA TGTACGTGTG GGGAGGTAAG AAACGGGACA GTTTGCGTCT 3480

CAATTCATTT GAAGACATAA GAAAGCAAAA TGTTCCTTGC CACATTTAAG GTAGTATGGA 3540

GAAACATGTC CCACAGTGGC CTTAAATATC ACTCTGAGCT CGAGTCTTGT GGTGGCTCAT 3600

GAACCATGGA GGACCTAGAG GTTCGAAGGG CAATTGACGC TTATCAAATG CCCTTATGTG 3660

CCAAGCACTG GGACTGGCCG ATTGGCATAC AAACCTAATT TAATTCTCGC AGGGAATGCA 3720

CGACACAGTT GATACCAGCC CATTTGACAG CCTGAGGACA TGTGAGTTGC TAAACCACCT 3780

CCTAAAGGCA ATGCAGCTTC TAAGTGGCAG AGTTTAGGAT TGAACGAGAA TTTGCCTATT 3840

TCAAAGTTTG TCCCCTCTCC TTGATGGTCT GTGCCTCCCC TGTCAAAGTC CAAAGGCTGA 3900

TTAGAAATTG AACATCATTA GCCAAAGCTG ATCAACAGCA GAGCCCCCAC TTGCAGATGG 3960

GAATGGTGAG AGAGGGAGAC TGAAACACTT TTTTCTTGGC CTTTCAGGGT TTAGAATCCA 4020

AGCTTAAGTT TCTGCCTTCC TGTCCCTTGT GTAGTGGTTG AGGACATGGA CTGAGCCCAT 4080

GCTCCAGATG GTATTTCTCC TCCAGTGCTC TCCCATCCAG CCCCCAGCCA ACTCTGGGTG 4140
CCATGAATGG GACTACGTCG GCTTTTACAG ACAGTTGTCT CCTCAGAGAC CGTTACAGTG 4200
CCTGACTCAC AGTAGGTGCT CAGTAAAAAG TGTTAAATGA ATGAATGGGC CTAGGTTTGT 4260
GTCCTGGGTC TATCATTCTC CAGCTGCCTA AGTTTGGGAA ATTGGCCTCT TGGAATCTCA 4320
GTCCCTCCCC TACAAAAGGG CAGCAATGAT TGTACTTTAT AGTTTCTAGT AGCTAATGAG 4380
ATAGCAACAG ATACTACAGA GGGCTCAGGA AATGCTACTG GTTATTATTA TTATTTTTTA 4440
TTTTATTTAT TTTTTGGGAG ACGGGGTCTT GCTCTATTAT CCAGGCCTGG GGTGGAGAGG 4500
CTCAATCAGA GCTCACTGCA GGTCCTCAAG CAATCCACCC ACTTCACCTC CTGAGTAGCC 4560
GGGACCACAG GCTGGTGCCA CCATGCCTGG CTTTTTTTTT TTTTTTAAAC TTAAAAAACA 4620
TAGGCGGCTC CCTATGTTGC CCAGGCTGGT CTCAAACTCC TGGACTGAAG CGATCCTCCT 4680
GCCTTATCCT CACAAAGTGC TGGGATTGCA GGCATGAGCC ACCACACCTG GCCTATGTTT 4740
AATATTATTG ATAATTCACC TCCTCACCTT CAATGCCTTC TTGCCTAGAG GAGGAGGCAG 4800
GTGAGCCCTT TCTAGTCCCC AGATAAGGTC CTCCAGCAGA TTCCTGAGGG ACCCACTTCC 4860
AGGCACAGCC CCTCATCTCC CTCTCCCTAC GAGAAGCTGA AGGAGTTCAG CTGCCAGCCT 4920
GTGTTGAACA GCTACCAGTT CCACATCGTC AATGGCGCAG TGGTTTGTGA GTAGCCTTTT 4980
CTGTATGGAA ATGTCTTTTA ACCTGGGCCT TTCCTTAACG TTCACCTCCT CTTTGACCCA 5040
GAGATCTTTT AGAAAATGAA ATGCTTCCAA GTGCTTGGAA GGAGATATTC CTGAGCTTTC 5100
TCCTGATGCT CCAGAGCTTC TCAGAGTGTC CGTGCTCATC CTGCCCTGGT CTCTCCCACC 5160
CATGAGTGTA CCTCCTGAAC TCTCTGGGGG CCCAGAGCCT GGCAGATAGT ACATGCTCAG 5220
TAAATACTTG TTCACTTGAG CTAATCTTGA AGCTTCCCTT GACAACTGCT GCTGTTGAGA 5280
ACATGTTTCC TTGTTTCTGT GATTTTGTTA ACAAAACGGC TCAGCTGTCT TCCAGTTGGA 5340
CAAATATTTA TTAAGGGCGA CTGCATGCCA AGCACTAAGA TAGGTGCTGC CAGGGCCACA 5400
AAAGCAAATA GGTGGGAAGG GAAGGGGGAC TCACATGTTA CTGAGACCAT TCAAGGAGCC 5460
ATGTGGGCAA GTGGATCAGT GCCCTTCACA TGGGGCGTGG CCTGGCATCC GGAGCGTGTT 5520
CTGCGGCTGG TAGGGTATGG GTATGTGCAG GGCAATCCTG GCCTAGACAG CAGGCACATT 5580
TGGAGGCACG GGACAGTAGT CTTTCGTGAG CACCATCCTT TCCAGCATAG CCAGGGTGGA 5640
TCCTGGGGTC CTGGGCTGGG AGGGTGAAGA GCAACAAATA AAGAAGTGGC TTCTTGGCCG 5700
GGCGCGGTGG CTCACGCTTG TAATCCCAGC ACTTTGGGAG GCCGAGGCGG GCGGATCACG 5760
AGGTCAGGAG ATCGAGACCA TCCTGGCTAA CACGGTGAAA CCCCGTCTCT ACTAAAAATA 5820
CAAAAAAAAT TAGCCGGGCG TGATGGTGGG CGCCTGTAGT CCCAGCTACT CGGGAGGCTG 5880

AGGCAGGAGA ATGGCGTGAA CCCGGGAGGC GGAGCTTGCA GTGAGCCGAG ATTGCGCCAC 5940
TGCACTCCCG CCTGGGCCAC AGAGCGAGAC TCCGTCTCAA AAAAAAAAAA AAAAAAAAAG 6000
AAGAAGTGGC TTCTTATAGT GTGTGGCTCA CTTCCTGCCT GGCCTCGTGG GGTTGCATGA 6060
ATCACTTTCC TTCCCAGGTG TATTTATTCA GAGCTGTGAG TGCACCTTGG AGTTCCTCTG 6120
TTTCCTCCTG AGGTCAGGGA ACTACCACCT CTCTGCCACT CATCCCCTAT GGCGGGAGAT 6180
ACATCCTCCA TCCCGTAGTG GGTTCCAGGG CTCAGAACCC TGGTACTCCT GAGCTCCCCA 6240
ACCCACCACT TCAGCTCAGC ACACACCAAT ACCCAGAGTT AGGACTGTGA GGTCTCCCTG 6300
GCACCAGCTG TGTGGGTTGG GGGCTCGGAC CCCTGCACCG GGAGGACCTG CCTCAGCTCT 6360
TGGCCTGCCC TGCCCACTGC CACCAGCACG TGGTTGACAG GGAAAGAACC CCCTTTTGTT 6420
CCCCACGTGA GCTCAAGCAA TCCACCCACT TCAGCCTCCT GAGTAGCTGG GATTACAGGT 6480
GCCCACTGCC ATGCTTGACT AATTTTTTGT ATTTTTAATA GAGACGGGGT TTCACCATCT 6540
TGGCCAGCTC AGCACACACC AATACCCAGA GTTAGGACTG TGAGGTCTCC CTGGCACCAG 6600
CTGTGTGGGT TGGGGGCTCG GACCCTGCAC CGGGAGACCT GCCTCAGCTC TTGGACTGCC 6660
TGCCACTGCC ACCAGCACGT GTTGACAGGG AAAGAACCCC TTTTGTTCCC ACGTGAGCTC 6720
AAGGAGACTT CCCTGAGTTG GAGCTCTCTG GTGTGGTCCT TCTCAGGCCT AAAGCAAAGT 6780
GTCTTTTCTG TGACACCTCC AAGGCCATGT TCAGGAGAGG GGAAGGGATC AGGGCCTGGT 6840
GGGAGGGATG GGGAGAGGGG ACTGGAGAAG GTGGCCTCCA GGGATCGAGT TTCCCATGGC 6900
CTCTTCCCAC CTGTCTTTGC CACAGGGGTG GGGACACCTG GCTGGCCCAG CCCAAGCCTC 6960
CACCCTGGGC TCCTGTGGGC TGGCTGCACT CGCCAGGGCT GGCCTAGGCT CTCTGCACCC 7020
AGGGAAGCTT CTCTATTCAA TGCTCTTCAC CCTCCCAGCC CAGGACCCCA GGAGATGAGG 7080
GAGAGTGGAG CAAAGGTTGA GGAGCAGAGG CTGGAGCCCC AGGCAGTGGC ACTGCTGGGC 7140
AGTGGTGGGA GGTGCCAGCC AGGGCTGGGA GTTGGACCCG AAAGTACGTG GCCTGGGCTG 7200
TACTTTCTTC CCACGTTGCC CCTTCAGAGC AGAAGCAGCC AGTTGCTCCT GAAGCCTTGA 7260
CCAGGGCTCC TGAGTCCAGA GCCTTGCTCA GGGCACTAGC GTGGGAGGAG GCTTCCGCAT 7320
CAGTACAGGG CATCAGCACC CGCCTCCTCA GCTGACCCAG CCCCGTGAGG ACCCAGGCCC 7380
AGCCCCCTGT CATCCCCACC CCCACCTTGC CAAGCCCCTG CCCCCAGGAG CAGGGCTGAG 7440
AGCGAGGTGA TCTGGGTTCT AATCCAGAGT CTGCTGCTGA CATGTGCTGA GCCCCAGGCC 7500
CATTGGTTTA CTTGCCCCAG TATTGAGCGA GCATCCACTG GGTACCCGCC CAGTGCCGGT 7560
GCTGTGCCAG GGGCCGGGGC ACAGAATAAA GCAGACCCGT CCCTGCTCTT CTGGCATTCA 7620
CAGTCTTGTG GAAACTCCAG ACTGAAAGTG CCCTTAGAGA TTATCCAGAT CAGCCCCTCC 7680

TTGTAGCAAT GAAGAGACTG AGACCCACAG AGGGGATGAG TTTGATCCAA GAAACAGACA 7740
AGATTAAGAT GCATGTGTCT TGAACCTTTT CAGTGCTCTG GAACATACCG TCTGGCCGGA 7800
GTTGTCTGGG CTTTGGTTTT CCCATCCATG AAATGGGTAC AATAACAACA GCTATAGTGT 7860
ATGAGCCTCT GTGATAGATG CTGTACGCAC AGCACCTGAA CTCACATGAT AAACCACTGA 7920
GGTGAGCATT ATCTCCCATT ATCAAGGAGG ACCCTGGGGC TCAGAGAGGT TAAGCACGAT 7980
GCCAAGGCCA CACAGCCAGG GAAAGAAGAG TTGGAATTCA AACCCCGGGT GCCCTGTCTC 8040
ACACTAGCTT CCCCTGTGGA GGGTGCTGGT GTGTGCATGA TTGGAGGCCC TCACACAGTG 8100
TAAGTCTCAG GATCTGCAGC AAACTGGTCA GAATGCTCTG CCCTGGCCCA GGGAAGGAAA 8160
GAGGGGCAGA TGGAGTTTGC TTCGCTGTAA GGCCCCGGAG CTTTGTGTTC CTGCTGAGAA 8220
GCCTCAGAGT CGGGCAACAC TGGGTCTAAT TCCAGCTCCA CCCCTTGTAT TAATAGCTGG 8280
GCCTTAATCT CCTCATCTGT AAAATGGAGA GAATCGTCGC CTGTACTTCA TAAGGCTGCT 8340
GGAAGGATTA GCTAAAGCAA CCCAGCTACA GTGGCTGGCC TACAGTAGGT GCTTCATTAA 8400
TGCCCTTCCT TTTAGATGTG GAAATTCCTC TTTTTGTCCA AGTTTTCTTT TCCTCTTTGC 8460
TTACGGCACT GGGATTTTCT TTATTACTGT TTCTTTGAAG AGTCCGCTCT GTACTTGTGC 8520
CCACGGCTAT GGTCAGTAAC CCCTTATGGA ATAAAACCCC TTTCCTGGCC AGGTGTGGTG 8580
GCTCATACCT GTAATCCCAG CACTCTGGGA GGCTGAGGCG GGAGGATCAC TTGAGCCCAG 8640
GAGTTCGAGA CCAGCCTGGG CAACACAGTG AGACCCCTGT CTCTACTAAA CATACAAACA 8700
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GCCTGGGCAA CAGAGTGAGA CCCTACCTCA AAAAGAAAGC AACAACAGAA AACCTATTTC 8880
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GCCTCCCATG AGTTCTGAAA TCCTGCACCC AGGCCTGGTC CCAGTTGCCT AGCAACCGGG 9000
GGCTGCTCTG GGATGCAGTA GGTAAGCAGG GGAGGGAGAG GAAGAAAACA ACTTGGTCTG 9060
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CAGCAGTGAC ACCCCCCTCC GCCCACATGC ACATACATGT GTGGTACAGG GAGGACCCGG 9300
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CTCTGTGTCT GTGTGCCTCG ACTCTCCCCC ACCGACTAGA AAGGTCCGTG AGAGCAAGGA 9600
GCAAGCCTGT CTTGTTTGAG GGCACTGGTT CTCATAGAGC CACAGGGAAT GATGCCCCTG 9660
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GCAAGGTAGT CAGCAAAGAG CCTTTACTGC ATGTTGGGGT CAGAAGATCA GCAATAAGGA 10020
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ACAGGCTGCA AAATGACAGC GTTTGGGGGT CAGGAGACAG AAGGGTGCCT GCTTTAGGTG 10200
GTCGAAGAAG GCCTCTCTGG GGAGGTGGCA TTTGGTCTGA GACCTCAGGG CCAATGTGCT 10260
AGGAGCAGAG GAGCCTTGGG GAAGAATGGA GATGAGGTTG GACAGGATGA GACACGTGCC 10320
TTCTATGTCA ATGGCAAGGG AGTCATTGGA GCATGTGAAG CAGAGGATGC TCTACTTTTG 10380
CCCCAGAAAG ATCACTCTGG CTACAGTGCA GAGAAAGAAG AGAGTCAAGG AGGAAAGAAG 10440
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GGTGACCAAT GGATGGAAGT GTCAGATGTC GCATGCTGTC GGTAGGTCAG GGCCGACAGG 10560
ACCTGTCGAT GGGTTCAGCG TGGGGTGTGA AGGAACACAG GCTGCACCCC AGCTCCTGGC 10620
CTGAGTGGCT GTAGATAGTG GCACCAAATA CTGAGCTCGT GAAGATGGGG GAGAGCTGAT 10680
GATGAAGACA GCAAGAGTTT GGTGTGAGTC ACCTTGAGTT TGAGACACGT GTCAGACATG 10740
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CTGCAGCGGA GAAGTTGGGA GTATTCAGAG TTCTGACACT GACCAAGAAC ACCCCTCAGA 10860
GAATTCAGAG ACAACCAGGG CTGAGGCGAG GGGCTTAGAC TGGGGCCTGG GACAGCCACA 10920
GGCAGGAATG CAGACTTGCT GCCTCTTCTT ATTTGTGGAG ATGTAGTTCA TGCAGCAAGA 10980
AAGTCATTCC AAAGCCCTCC TTTCCTTTCT TCATGCCTCA GTTTCTCCAT TAGCACATTA 11040
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GCCACAGAAG TGACAGTCCA CTGCAAAAGA CTGCTGCTCC AGATCAGTTC TGGAAGGCCT 11220
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GGAAGACCAA ATGAGAAAGG GAGAGGGGGC AGGGAGAAAG GAAGGAGAGC TAGAGACTTG 11340

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GACAAGCAAT CCGTGCACTG CTTCAAAGAG AGCCTGCCCA CCTATGAGAA AAACTTCAAG 13980
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ATCCCCAACA GGCCCCTGTT CTTCCACTGG GAGGGAGGAC AAAATGTCTC CCGCAGGGCA 14160
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CAGGAGGGGA GAAGCCCCAG GATCTACCCC AAACTCTCTC TTCACCCCCA CTGCAAACCG 14460
GGACACAGAG CAGACTTGAG CGCCAGGCCC ATGCCCAGCT CTAGCTGGCA ACAAAGCCAC 14520
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GACCAAAACA ACTGTCCAGC TGAGCCCAGG CTAAACTGCC AACATGCAGA ATTGTGAGCT 14940

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TTCAGGGAAC ACACATTTAT TGAGCACTTG CTATGTGCCA GGCACAGTGC CAGGCAGTAG 15240

GGATCCAGAT ATTTAAAGAA AACAAACAAA AATCAGGTCC AAAACTCCTG GGGAGAATGC 15300

TGAGAGTGGT ATCAGCTTTT AGGAATTC 15328

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Met Lys Leu Leu Leu Leu Ala Ala Leu Leu Thr Ala Gly Val Thr Ala
1 5 10 15

His Ser Ile Ser Thr Arg Ala Val Trp Gln Phe Arg Asn Met Ile Lys
20 25 30

Cys Thr Ile Pro Gly Ser Asp Pro Leu Arg Glu Tyr Asn Asn Tyr Gly
35 40 45

Cys Tyr Cys Gly Leu Gly Gly Ser Gly Thr Pro Val Asp Asp Leu Asp
50 55 60

Arg Cys Cys Gln Thr His Asp His Cys Tyr Asn Gln Ala Lys Lys Leu
65 70 75 80

Glu Ser Cys Lys Phe Leu Ile Asp Asn Pro Tyr Thr Asn Thr Tyr Ser
85 90 95

Tyr Lys Cys Ser Gly Asn Val Ile Thr Cys Ser Asp Lys Asn Asn Asp
100 105 110

Cys Glu Ser Phe Ile Cys Asn Cys Asp Arg Gln Ala Ala Ile Cys Phe
115 120 125

Ser Lys Val Pro Tyr Asn Lys Glu Tyr Lys Asp Leu Asp Thr Lys Lys
130 135 140

His Cys
145

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met Lys Val Leu Leu Leu Leu Ala Val Val Ile Met Ala Phe Gly Ser
1 5 10 15

Ile Gln Val Gln Gly Ser Leu Leu Glu Phe Gly Gln Met Ile Leu Phe
20 25 30

Lys Thr Gly Lys Arg Ala Asp Val Ser Tyr Gly Phe Tyr Gly Cys His
35 40 45

Cys Gly Val Gly Gly Arg Gly Ser Pro Lys Asp Ala Thr Asp Trp Cys
50 55 60

Cys Val Thr His Asp Cys Cys Tyr Asn Arg Leu Glu Lys Arg Gly Cys
65 70 75 80

Gly Thr Lys Phe Val Thr Tyr Lys Phe Ser Tyr Arg Gly Gly Gln Ile
85 90 95

Ser Cys Ser Thr Asn Gln Asp Ser Cys Arg Lys Gln Leu Cys Gln Cys
100 105 110

Asp Lys Ala Ala Ala Glu Cys Phe Ala Arg Asn Lys Lys Ser Tyr Ser
115 120 125

Leu Lys Tyr Gln Phe Tyr Pro Asn Lys Phe Cys Lys Gly Lys Thr Pro
130 135 140

Ser Cys
145

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 148 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Lys Leu Leu Val Leu Ala Val Leu Leu Thr Val Ala Ala Ala Asp

```
1               5                   10                  15

Ser Gly Ile Ser Pro Arg Ala Val Trp Gln Phe Arg Lys Met Ile Lys
            20                  25                  30

Cys Val Ile Pro Gly Ser Asp Pro Phe Leu Glu Tyr Asn Asn Tyr Gly
        35                  40                  45

Cys Tyr Cys Gly Leu Gly Gly Ser Gly Thr Pro Val Asp Glu Leu Asp
    50                  55                  60

Lys Cys Cys Gln Thr His Asp Asn Cys Tyr Asp Gln Ala Lys Lys Leu
65                  70                  75                  80

Asp Ser Cys Lys Phe Leu Leu Asp Asn Pro Tyr Thr His Thr Tyr Ser
                85                  90                  95

Tyr Ser Cys Ser Gly Ser Ala Ile Thr Cys Ser Ser Lys Asn Lys Glu
            100                 105                 110

Cys Glu Ala Phe Ile Cys Asn Cys Asp Arg Asn Ala Ala Ile Cys Phe
        115                 120                 125

Ser Lys Ala Pro Tyr Asn Lys Ala His Lys Asn Leu Asp Thr Lys Lys
    130                 135                 140

Tyr Cys Gln Ser
145
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 144 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Met Lys Thr Leu Leu Leu Leu Ala Val Ile Met Ile Phe Gly Leu Leu
1               5                   10                  15

Gln Ala His Gly Asn Leu Val Asn Phe His Arg Met Ile Lys Leu Thr
            20                  25                  30

Thr Gly Lys Glu Ala Ala Leu Ser Tyr Gly Phe Tyr Gly Cys His Cys
        35                  40                  45

Gly Val Gly Gly Arg Gly Ser Pro Lys Asp Ala Thr Asp Arg Cys Cys
    50                  55                  60

Val Thr His Asp Cys Cys Tyr Lys Arg Leu Glu Lys Arg Gly Cys Gly
65                  70                  75                  80

Thr Lys Phe Leu Ser Tyr Lys Phe Ser Asn Ser Gly Ser Arg Ile Thr
                85                  90                  95
```

Cys Ala Lys Gln Asp Ser Cys Arg Ser Gln Leu Cys Glu Cys Asp Lys
100 105 110

Ala Ala Ala Thr Cys Phe Ala Arg Asn Lys Thr Thr Tyr Asn Lys Lys
115 120 125

Tyr Gln Tyr Tyr Ser Asn Lys His Cys Arg Gly Ser Thr Pro Arg Cys
130 135 140

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ala Val Trp Gln Phe Arg Lys Met Ile Lys Cys Val Ile Pro Gly Ser
1 5 10 15

Asp Pro Phe Leu Glu Tyr Asn Asn Tyr Gly Cys Tyr Cys Gly Leu Gly
20 25 30

Gly Ser Gly Thr Pro Val Asp Glu Leu Asp Lys Cys Cys Gln Thr His
35 40 45

Asp Asn Cys Tyr Asp Gln Ala Lys Lys Leu Asp Ser Cys Lys Phe Leu
50 55 60

Leu Asp Asn Pro Tyr Thr His Thr Tyr Ser Tyr Ser Cys Ser Gly Ser
65 70 75 80

Ala Ile Thr Cys Ser Ser Lys Asn Lys Glu Cys Glu Ala Phe Ile Cys
85 90 95

Asn Cys Asp Arg Asn Ala Ala Ile Cys Phe Ser Lys Ala Pro Tyr Asn
100 105 110

Lys Ala His Lys Asn Leu Asp Thr Lys Lys Tyr Cys Gln Ser
115 120 125

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Asn Leu Val Asn Phe His Arg Met Ile Lys Leu Thr Thr Gly Lys Glu
1 5 10 15

Ala Ala Leu Ser Tyr Gly Phe Tyr Gly Cys His Cys Gly Val Gly Gly
20 25 30

Arg Gly Ser Pro Lys Asp Ala Thr Asp Arg Cys Cys Val Thr His Asp
35 40 45

Cys Cys Tyr Lys Arg Leu Glu Lys Arg Gly Cys Gly Thr Lys Phe Leu
50 55 60

Ser Tyr Lys Phe Ser Asn Ser Gly Ser Arg Ile Thr Cys Ala Lys Gln
65 70 75 80

Asp Ser Cys Arg Ser Gln Leu Cys Glu Cys Asp Lys Ala Ala Ala Thr
85 90 95

Cys Phe Ala Arg Asn Lys Thr Thr Tyr Asn Lys Lys Tyr Gln Tyr Tyr
100 105 110

Ser Asn Lys His Cys Arg Gly Ser Thr Pro Arg Cys
115 120

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Gly Leu Leu Asp Leu Lys Ser Met Ile Glu Lys Val Thr Gly Lys Asn
1 5 10 15

Ala Leu Thr Asn Tyr Gly Phe Tyr Gly Cys Tyr Cys Gly Trp Gly Gly
20 25 30

Arg Gly Thr Pro Lys Asp Gly Thr Asp Trp Cys Cys Trp Ala His Asp
35 40 45

His Cys Tyr Gly Arg Leu Glu Glu Lys Gly Cys Asn Ile Arg Thr Gln
50 55 60

Ser Tyr Lys Tyr Arg Phe Ala Trp Gly Val Val Thr Cys Glu Pro Gly
65 70 75 80

Pro Phe Cys His Val Asn Leu Cys Ala Cys Asp Arg Lys Leu Val Tyr
85 90 95

Cys Leu Lys Arg Asn Leu Arg Ser Tyr Asn Pro Gln Tyr Gln Tyr Phe

100 105 110

Pro Asn Ile Leu Cys Ser
115

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Ala Val Trp Gln Phe Arg Asn Met Ile Lys Cys Thr Ile Pro Gly Ser
1 5 10 15

Asp Pro Leu Arg Glu Tyr Asn Asn Tyr Gly Cys Tyr Cys Gly Leu Gly
20 25 30

Gly Ser Gly Thr Pro Val Asp Asp Leu Asp Arg Cys Cys Gln Thr His
35 40 45

Asp His Cys Tyr Asn Gln Ala Lys Lys Leu Glu Ser Cys Lys Phe Leu
50 55 60

Ile Asp Asn Pro Tyr Thr Asn Thr Tyr Ser Tyr Lys Cys Ser Gly Asn
65 70 75 80

Val Ile Thr Cys Ser Asp Lys Asn Asn Asp Cys Glu Ser Phe Ile Cys
85 90 95

Asn Cys Asp Arg Gln Ala Ala Ile Cys Phe Ser Lys Val Pro Tyr Asn
100 105 110

Lys Glu Tyr Lys Asp Leu Asp Thr Lys Lys His Cys
115 120

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Ser Leu Leu Glu Phe Gly Gln Met Ile Leu Phe Lys Thr Gly Lys Arg
1 5 10 15

Ala Asp Val Ser Tyr Gly Phe Tyr Gly Cys His Cys Gly Val Gly Gly
20 25 30

Arg Gly Ser Pro Lys Asp Ala Thr Asp Trp Cys Cys Val Thr His Asp
35 40 45

Cys Cys Tyr Asn Arg Leu Glu Lys Arg Gly Cys Gly Thr Lys Phe Val
50 55 60

Thr Tyr Lys Phe Ser Tyr Arg Gly Gly Gln Ile Ser Cys Ser Thr Asn
65 70 75 80

Gln Asp Ser Cys Arg Lys Gln Leu Cys Gln Cys Asp Lys Ala Ala Ala
85 90 95

Glu Cys Phe Ala Arg Asn Lys Lys Ser Tyr Ser Leu Lys Tyr Gln Phe
100 105 110

Tyr Pro Asn Lys Phe Cys Lys Gly Lys Thr Pro Ser Cys
115 120 125

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 130 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Ser Phe Trp Gln Phe Gln Arg Met Val Lys His Ile Thr Gly Arg Ser
1 5 10 15

Ala Phe Phe Ser Tyr Tyr Gly Tyr Gly Cys Tyr Cys Gly Leu Gly Gly
20 25 30

Arg Gly Ile Pro Val Asp Ala Thr Asp Arg Cys Cys Trp Ala His Asp
35 40 45

Cys Cys Tyr His Lys Leu Lys Glu Tyr Gly Cys Gln Pro Ile Leu Asn
50 55 60

Ala Tyr Gln Phe Ala Ile Val Asn Gly Thr Val Thr Cys Gly Cys Thr
65 70 75 80

Met Gly Gly Gly Cys Leu Cys Gly Gln Lys Ala Cys Glu Cys Asp Lys
85 90 95

Leu Ser Val Tyr Cys Phe Lys Glu Asn Leu Ala Thr Tyr Glu Lys Thr
100 105 110

Phe Lys Gln Leu Phe Pro Thr Arg Pro Gln Cys Gly Arg Asp Lys Leu
115 120 125

His Cys

130

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Leu Leu Glu Leu Lys Ser Met Ile Glu Lys Val Thr Gly Lys Asn
1 5 10 15

Ala Val Lys Asn Tyr Gly Phe Tyr Gly Cys Tyr Cys Gly Trp Gly Gly
20 25 30

His Gly Thr Pro Lys Asp Gly Thr Asp Trp Cys Cys Arg Met His Asp
35 40 45

Arg Cys Tyr Gly Leu Leu Glu Glu Lys His Cys Ala Ile Arg Thr Gln
50 55 60

Ser Tyr Asp Tyr Arg Phe Thr Gln Asp Leu Val Ile Cys Glu His Asp
65 70 75 80

Ser Phe Cys Pro Val Arg Leu Cys Ala Cys Asp Arg Lys Leu Val Tyr
85 90 95

Cys Leu Arg Arg Asn Leu Trp Ser Tyr Asn Arg Leu Tyr Gln Tyr Tyr
100 105 110

Pro Asn Phe Leu Cys
115

The present invention may, of course, be carried out in other specific ways than those herein set forth without departing from the spirit and essential characteristics of the invention. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive and all changes coming within the meaning and equivalency range of the appended claims are intended to be embraced herein.

Having described our invention, we claim:

1.) A substantially pure or isolated low molecular weight PLA_2 enzyme having phospholipase activity, said enzyme being free of disulfide bridges between cysteine amino acids 11 and 77 and an elapid loop, said enzyme having at least seventeen amino acids in its sequence which are identical to those amino acids conserved in Type II PLA_2 enzymes having phospholipase activity.

2.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme having only 12 cysteine amino acids residues in its mature sequence.

3.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme having only 16 cysteine amino acid residues in its mature sequence.

4.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme having a molecular weight of about 14KD.

5.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme having an amino acid sequence set forth in FIG. 3 or an equivalent fragment thereto or an active fragment thereof.

6.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme having an amino acid sequence set forth in FIG. 11 or an equivalent fragment thereto or an active fragment thereof.

7.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme having an amino acid sequence set forth in FIG. 12 or an equivalent fragment thereto or an active fragment thereof.

8.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme having an amino acid sequence encoded for by the nucleotide sequence of FIG. 19 or an equivalent fragment thereto or an active fragment thereof.

9.) A PLA_2 enzyme of claim 2, said PLA_2 enzyme having an amino acid sequence which includes the following prepeptide amino acid sequence MDLLVSSGMKGIAVFLVFIFC.

10.) A PLA_2 enzyme of claim 2, said PLA_2 enzyme having an amino acid sequence which includes the following propeptide amino acid sequence WTTSTLS.

11.) A PLA_2 enzyme of claim 2, said PLA_2 enzyme having the following features:

a.) a phenylalanine residue conserved at position 5 in the mature sequence;

b.) a methionine residue conserved at position 8 in the mature sequence;

c.) a histidine residue conserved at position 48 and an aspartic acid residue at position 49 in the mature sequence;

d.) a valine residue conserved at position 9 in the mature sequence; and

e.) being free of alanine residues at positions 102 and 103 in the mature sequence.

12.) A PLA_2 enzyme of claim 2, said PLA_2 enzyme having a YGCYCG Ca^{2+} binding loop.

13.) A PLA_2 enzyme of claim 3, said PLA_2 enzyme having an amino acid sequence which includes a prepeptide amino acid sequence selected from a group consisting of MKGLLPLAWFLACSVPAVQG and MKRLLTLAWFLACSVPAVPG.

14.) A PLA_2 enzyme of claim 3, said PLA_2 enzyme having the following features:

a.) an isoleucine residue conserved at position 9 in the mature sequence;

b.) a methionine residue conserved at position 8 in the mature sequence;

c.) a histidine residue conserved at position 48 and an aspartic acid residue conserved at position 49 in the mature sequence;

d.) a leucine residue conserved at position 5 in the mature sequence; and

e.) being free of alanine residues at positions 102 and 103 in the mature sequence.

15.) A PLA_2 enzyme of claim 3, said PLA_2 enzyme having a YGCYCG Ca^{2+} binding loop.

16.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme being a Type III PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof.

17.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme being a TYPE IV PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof.

18.) A PLA_2 enzyme of claim 1, said PLA_2 enzyme further including a COOH-terminal amino acid extension.

19.) A PLA_2 enzyme of claim 18, said PLA_2 enzyme being a Type III PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof, said Type III PLA_2 enzyme having an about seven amino acids COOH-terminal extension.

20.) A PLA_2 enzyme of claim 19, said seven amino acids COOH-terminal extension having the following amino acid sequence GRDKLHC, said Type III PLA_2 enzyme being a rat Type III PLA_2 enzyme.

21.) A PLA_2 enzyme of claim 18, said PLA_2 enzyme being a Type IV PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof, said type IV PLA_2 enzyme having an about one amino acid COOH-terminal extension.

22.) A PLA_2 enzyme of claim 21, said one amino acid COOH-terminal extension having a serine amino acid COOH-terminal extension, said Type IV PLA_2 enzyme being a human Type IV PLA_2 enzyme.

23.) A substantially pure or isolated nucleotide sequence coding for a polypeptide having phospholipase activity, the polypeptide having no disulfide bridges between cysteine amino acids 11 and 77 and no elapid loops.

24.) A nucleotide sequence of claim 23, the polypeptide having the amino acid sequence set forth in FIG. 3 or an equivalent fragment thereto or an active fragment thereof.

25.) A nucleotide sequence of claim 23, the polypeptide having the amino acid sequence set forth in FIG. 11 or an equivalent fragment thereto or an active fragment thereof.

26.) A nucleotide sequence of claim 23, the polypeptide having the amino acid sequence set forth in FIG. 12 or an equivalent fragment thereto or an active fragment thereof.

27.) A nucleotide sequence of claim 23, the polypeptide having the amino acid sequence encoded by the nucleotide sequence set forth in FIG. 19 or an equivalent fragment thereto or an active fragment thereof.

28.) A nucleotide sequence of claim 23, the polypeptide sequence having:

a.) a phenylalanine residue conserved at position 5 in the mature amino acid sequence;

b.) a methionine residue conserved at position 8 in the mature amino acid sequence;

c.) a histidine residue conserved at position 48 and an aspartic acid residue conserved at position 49 in the mature amino acid sequence; and

d.) being free of alanine residues at positions 102 and 103 in the mature amino acid sequence.

29.) A nucleotide sequence of claim 28, the polypeptide sequence having only 16 cysteine residues in its mature amino acid sequence.

30.) A nucleotide sequence of claim 29, the polypeptide sequence including the following prepeptide amino acid sequence MDLLVSSGMKGIAVFLVFIFC.

31.) A nucleotide sequence of claim 23, the polypeptide sequence having:

a.) an isoleucine residue conserved at position 9 in the mature amino acid sequence;

b.) a methionine residue conserved at position 8 in the mature amino acid sequence;

c.) a histidine residue conserved at position 48 and an aspartic acid residue conserved at position 49 in the amino acid sequence;

d.) 12 cysteine residues in the mature amino acid sequence; and

e.) being free of alanine residues at position 102 and 103 in the mature amino acid sequence.

32.) A nucleotide sequence of claim 29, the polypeptide sequence including the following propeptide amino acid sequence WTTSTLS.

33.) A nucleotide sequence of claim 31, the polypeptide sequence including a prepeptide amino acid sequence selected from a group consisting of MKGLLPLAWFLACSVPAVQG and MKRLLTLAWFLACSVPAVPG.

34.) A nucleotide sequence of claim 23, the polypeptide being a Type III PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof.

35.) A nucleotide sequence of claim 23, the polypeptide being a Type IV PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof.

36.) A nucleotide sequence of claim 23, the polypeptide further including a COOH-terminal amino acid extension.

37.) A nucleotide sequence of claim 36, the polypeptide being a Type III PLA_2 enzyme or an equivalent fragment thereto or active fragment thereof, said Type III PLA_2 enzyme having an about seven amino acids COOH-terminal extension.

38.) A nucleotide sequence of claim 37, said seven amino acids COOH-terminal extension having the following amino acid sequence GRDKLHC, said Type III PLA_2 enzyme being a rat Type III PLA_2 enzyme.

39.) A nucleotide sequence of claim 36, the polypeptide being a Type IV PLA_2 enzyme or an equivalent fragment thereto or active fragment thereof, said Type IV PLA_2 enzyme having an about one amino acid COOH-terminal extension.

40.) A nucleotide sequence of claim 39, said one amino acid COOH-terminal extension being a serine amino acid COOH-terminal extension, said Type IV PLA_2 enzyme being a human Type IV PLA_2 enzyme.

41.) A recombinant DNA expression vector comprising:

a first DNA segment having a nucleotide sequence containing bases whose translated region codes for a PLA_2 enzyme selected from a group consisting of Type III and Type IV or an equivalent fragment thereto or an active fragment thereof; and

a second DNA segment heterologous to said first DNA segment wherein said first DNA segment is operably linked to said second DNA segment.

42.) A recombinant DNA expression vector of claim 41, said first DNA segment having the nucleotide sequence set forth in FIG. 3 or an equivalent fragment thereto or an active fragment thereof.

43.) A recombinant DNA expression vector of claim 41, said first DNA segment having the nucleotide sequence set forth in FIG. 11 or an equivalent fragment thereto or an active fragment thereof.

44.) A recombinant DNA expression vector of claim 41, said first DNA segment having the nucleotide sequence set forth in FIG. 12 or an equivalent fragment thereto or an active fragment thereof.

45.) A recombinant DNA expression vector of claim 41, said first DNA segment having the nucleotide sequence set forth in FIG. 19 or an equivalent fragment thereto or an active fragment thereof.

46.) A recombinant expression vector of claim 41, said vector being pCH10.

47.) A recombinant expression vector of claim 41, said vector being pR8-3'.

48.) A host transfected with said recombinant expression vector of claim 41.

49.) A host of claim 48, said host being a cell line.

50.) A host of claim 49, said cell line being a cell line designated as CpCH10-1D.

51.) A host of claim 49, said cell line being a cell line selected from a group consisting of CpCH10-1B, CpCH10-1C and CpCH10-2G.

52.) A host of claim 49, said cell line being a cell line designated as CpR8-3'.

53.) A cDNA encoding a phospholipase enzyme having phospholipase activity, said phospholipase enzyme being selected from a group consisting of Type III and Type IV, including equivalent fragments thereto and active fragments thereof.

54.) A cDNA of claim 53, said phospholipase enzyme being $RPLA_2$-8 or an equivalent fragment thereto or an active fragment thereof.

55.) A cDNA of claim 53, said phospholipase enzyme being $HPLA_2$-10 or an equivalent fragment thereto or an active fragment thereof.

56.) A cDNA of claim 53, said phospholipase enzyme being $RPLA_2$-10 or an equivalent fragment thereto or an active fragment thereof.

57.) A method of producing a PLA_2 enzyme selected from a group consisting of Type III and Type IV or an equivalent fragment thereto or an active fragment thereof, said method comprising:

a.) inserting a recombinant expression vector into a host by transfection, said recombinant expression vector having a nucleotide sequence containing bases whose translated region codes for the Type III or Type IV PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof;

b.) cultivating the transfected host; and

c.) expressing the Type III or Type IV PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof by the transfected host.

58.) A method of claim 57, said cultivating step comprises growing the host in a cell culture medium.

59.) A method of claim 57, said cultivating step comprises introducing the host into an animal.

60.) A method of claim 57, the host being an eukaryotic cell.

61.) A method of claim 57, the host being a prokaryotic cell.

62.) A method of expressing a Type III or Type IV PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof in an animal comprising:

introducing a nucleotide sequence containing bases whose translated region codes for the Type III or Type IV PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof; and

expressing the nucleotide sequence in the animal.

63.) A method of claim 62, said nucleotide sequence including the nucleotide sequence set forth in FIG. 3 or an equivalent fragment thereto or an active fragment thereof.

64.) A method of claim 62, said nucleotide sequence including the nucleotide sequence set forth in FIG. 11 or an equivalent fragment thereto or an active fragment thereof.

65.) A method of claim 62, said nucleotide sequence including the nucleotide sequence set forth in FIG. 12 or an equivalent fragment thereto or an active fragment thereof.

66.) A method of claim 62, said nucleotide sequence including the nucleotide sequence set forth in FIG. 19 or an equivalent fragment thereto or an active fragment thereof.

67.) A method of claim 62, said introduction step comprises introducing a recombinant expression vector into the animal, the recombinant expression vector having the nucleotide sequence.

68.) A method of claim 62, said introduction step comprises introducing the nucleotide sequence into the genome of an animal.

69.) A substantially pure or isolated antisense nucleotide sequence which has the ability to inhibit or interfere with expression of a gene or mRNA transcript encoding for a Type III PLA_2 enzyme or an amino acid sequence which is an equivalent thereto or an active fragment thereof.

70.) A substantially pure or isolated antisense nucleotide sequence which has the ability to inhibit or interfere with expression of a gene or mRNA transcript encoding for a Type IV PLA_2 enzyme or an amino acid sequence which is an equivalent thereto or an active fragment thereof.

71.) A substantially pure or isolated Type III PLA_2 enzyme, or an equivalent fragment thereto or an active fragment thereof, said PLA_2 enzyme having phospholipase activity which is significant at a pH of between about 7 and about 9 and at a calcium concentration of between about 0.3 mM and about 2 mM.

72.) A Type III PLA_2 enzyme of claim 71, said phospholipase activity progressively declining at a pH which is greater than about 9 and at a calcium concentration which is greater than about 2 mM.

73.) A substantially pure or isolated Type IV PLA_2 enzyme, or an equivalent fragment thereto or an active fragment thereof, said PLA_2 enzyme having phospholipase activity which is significant at a pH of between about 6.5 and about 7.5 and at a calcium concentration of between about 7 mM and about 100 mM.

74.) A Type IV PLA_2 enzyme of claim 73, said phospholipase activity progressively declining at a calcium concentration of greater than about 100 mM.

75.) A substantially pure or isolated nucleotide sequence having an internal ribosome binding site which allows for internal initiation of cap-independent mRNA translation, said nucleotid sequence including bases 116-720 designated in FIG. 3 or an equivalent fragment thereto or an active fragment thereof.

76.) A nucleotide sequence of claim 75, said nucleotide sequence being operably linked to a second nucleotide sequence heterologous to said nucleotide sequence.

77.) A nucleotide sequence of claim 76, said second nucleotide sequence containing bases whose translated region encodes for luciferase.

78.) A nucleotide sequence of claim 76, said second nucleotide sequence containing bases whose translated region encodes for a Type III PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof.

79.) A nucleotide sequence of claim 78, said second nucleotide sequence includes the nucleotide sequence set forth in FIG. 3 or an equivalent fragment thereto or an active fragment thereof.

80.) A nucleotide sequence of claim 78, said second nucleotide sequence includes the nucleotide sequence set forth in FIG. 19 or an equivalent fragment thereto or an active fragment thereof.

81.) A nucleotide sequence of claim 76, said second nucleotide sequence containing bases whose translated region encodes for a Type IV PLA_2 enzyme or an equivalent fragment thereto or an active fragment thereof.

82.) A nucleotide sequence of claim 81, said second nucleotide sequence includes the nucleotide sequence set forth in FIG. 11 or an equivalent fragment thereto or an active fragment thereof.

83.) A nucleotide sequence of claim 81, said second nucleotide sequence includes the nucleotide sequence set forth in FIG. 12 or an equivalent fragment thereto or an active fragment thereof.

84.) A nucleotide sequence of claim 86, a recombinant expression vector including said nucleotide sequence operably linked to said second nucleotide sequence heterologous to said nucleotide sequence.

Fig. 1

RPLA2-8 cDNA Structure

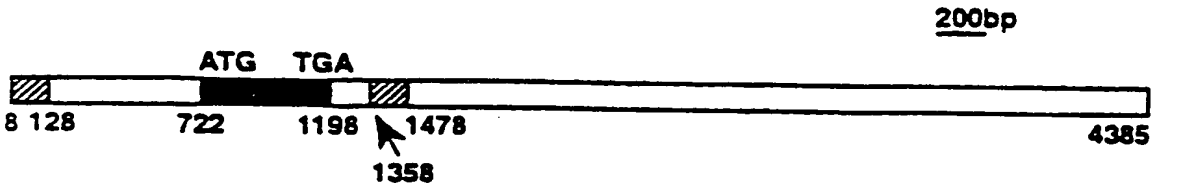

Fig. 2

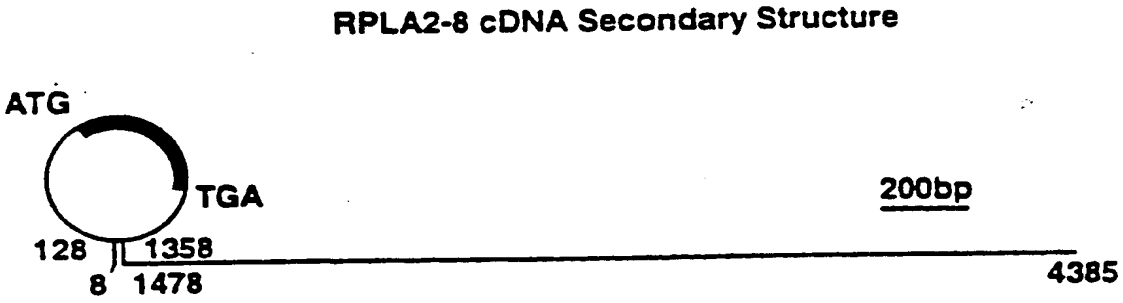

Fig. 3 RPLA2-8 cDNA and Derived Amino Acid Sequence

(1/5)

```
        10         20         30         40         50         60
GAATTCCGCCTCCACCTCTCAAATGCTGGGATTGCAGGATGTCCCCCCACCCCTGCTCCC
clone linker
        70         80         90        100        110        120
TTGTGTCCTTGCTTCCTGCTGCCGGAATGTATCACTTAATTGCCAGGTACCCATGGTCTG
                   Pla8-8 (primer)
       130        140        150        160        170        180
ATTCCAGGATAGAAGGGCGGGCGAGGGGGTTGGAGGAGAGGCCTCTATTATTTCCGCGGT

       190        200        210        220        230        240
CTGGCAGGCCTGGAAGCAAAGCTTCAAGTGCAGAAGGAGGAGTGTCGGGGAATGGCAGAA
                 Pla8-7 (primer)
       250        260        270        280        290        300
AAGGCTGGAACAGCAATGCAGACCTAGGTAAAGGGCACAGAGCTGAGGGAAGCTCCTGGG

       310        320        330        340        350        360
AGGCTCCCTGCAGCTCCTGCCTCTGCACATGACCCGGACTCCTTTTCTCTCTTTGGATCT

       370        380        390        400        410        420
GCGTCCAGGGACTGGCTTGTACACACCCCTCCCAGGAGACCCCTTGGCAGCTGCACACTC

       430        440        450        460        470        480
AGGCTCCATCCAAGTTGGCTCTGCCCCTGGGGAAGGCTGCTCAAAAGGCCTGGCTCCCAG

       490        500        510        520        530        540
TTTCTGGGGACCCACAGAGAGCCTCTCACCTCGCAGCTCAGCTCCATCCGCCTCCTGTGC

       550        560        570        580        590        600
CTGGCTGCGACCAGCTGGGTCTAACTATAGACAGTCAGCAACTTCAGCCACTTCACCGAG

       610        620        630        640        650        660
TTTCCCAACAGCTTTGAGATTTGGAAGCCGGAAGCCTGACTGCCTTCTCAGAAGCTACGG

       670        680        690        700        710        720
TCCACTACCTCAGCCATTCTGTTGGAGCTGAACTGGCAGATGAAGGTGAGACCCAGGCAC

       730        740        750        760        770        780
CATGGACCTCCTGGTCTCCTCAGGAATGAAGGGCATCGCTGTCTTCCTTGTCTTTATCTT
 MetAspLeuLeuValSerSerGlyMetLysGlyIleAlaValPheLeuValPheIlePh
  Rclo8-5' (primer)
       790        800        810        820        830        840
CTGCTGGACAACCTCCACCCTCAGCAGCTTCTGGCAGTTCCAGAGGATGGTCAAACACAT
eCysTrpThrThrSerThrLeuSerSerPheTrpGlnPheGlnArgMetValLysHisIl
                                Pla8-1 (primer)
       850        860        870        880        890        900
CACGGGGCGCAGCGCCTTCTTCTCCTATTACGGATATGGCTGCTACTGTGGGCTTGGGGG
eThrGlyArgSerAlaPhePheSerTyrTyrGlyTyrGlyCysTyrCysGlyLeuGlyGl

       910        920        930        940        950        960
CCGAGGGATCCCTGTGGACGCCACAGACAGGTGCTGCTGGGCTCATGACTGTTGCTACCA
yArgGlyIleProValAspAlaThrAspArgCysCysTrpAlaHisAspCysCysTyrHi
```

$RPLA_2$-8 cDNA sequence corresponds to SEQ ID NO:21: and Derived Amino Acid sequence corresponds to SEQ ID NO:22:.

FIG. 3 (2/5)

Pla8-2 (primer)

```
       970        980        990       1000       1010       1020
CAAGCTTAAGGAATATGGCTGCCAGCCCATCTTGAATGCCTATCAGTTTGCCATTGTCAA
sLysLeuLysGluTyrGlyCysGlnProIleLeuAsnAlaTyrGlnPheAlaIleValAs

      1030       1040       1050       1060       1070       1080
CGGGACCGTGACCTGTGGATGCACCATGGGTGGCGGCTGCTTGTGCGGGCAGAAAGCCTG
nGlyThrValThrCysGlyCysThrMetGlyGlyGlyCysLeuCysGlyGlnLysAlaCy

      1090       1100       1110       1120       1130       1140
TGAGTGTGACAAACTGTCTGTGTACTGCTTCAAGGAGAACCTGGCCACCTACGAGAAAAC
sGluCysAspLysLeuSerValTyrCysPheLysGluAsnLeuAlaThrTyrGluLysTh

      1150       1160       1170       1180       1190       1200
TTTCAAGCAGCTCTTCCCCACCAGGCCCCAGTGTGGCAGGGACAAACTCCATTGCTAGGC
rPheLysGlnLeuPheProThrArgProGlnCysGlyArgAspLysLeuHisCysEnd
                                                 Rclo8-3' (primer)
      1210       1220       1230       1240       1250       1260

      1270       1280       1290       1300       1310       1320
CTTCCCCTCCAAGAGTCCCCAGGCTCCTGCAGCTCAGCCTTGCTGTCTAGGGAGTGTCTT

      1330       1340       1350       1360       1370       1380
CTCAGGCATTAGGGGACCGGAGGTGGAGAATTCCTGCCCTGGAATCAGACCATGGGTACC

      1390       1400       1410       1420       1430       1440
TGGCAATTAAGTGATACATTCCGGCAGCAGGAAGCAAGGACACAAGGGAGCAGGGGTGGG

      1450       1460       1470       1480       1490       1500
GGGACATCCTGCAATCCCAGCATTTGAGAGGTGGAGGCAAGAGGTGGGGGGTAGCCTCCA

      1510       1520       1530       1540       1550       1560
CTATACGGTAAGTTCAAGGCTAACCTGAGCTACCTGAGACCTTGCCTTGAAAAAACTTTT

      1570       1580       1590       1600       1610       1620
TTAAAAAACGTTTAAAGGAAAAGAAAACAGAAAGACACGGGGACTGGGCTGAAAGGTACT

      1630       1640       1650       1660       1670       1680
CTCAAACCGATTTCCCAGGAAGAGCGGAGAGCCCCAGGTTCAGCTCCAGCCTGAACTCCC

      1690       1700       1710       1720       1730       1740
CCATACCCTCAGTCCTGGTCAGGATGTGTGTCTGACTGGGGAACCAAGTCCTCCACCCGG

      1750       1760       1770       1780       1790       1800
GTGGAGCTTAGCTGGGAACTACGCAGGTGTCCTAGAAAATACAGTCCTAAGAGCCTCACC

      1810       1820       1830       1840       1850       1860
CGGAGTCTCATCCCCATTTGCTCCAGGACTGACCTCTGTAAATCTTCCAGCAGGAAGCAG

      1870       1880       1890       1900       1910       1920
GCTGTACCCATCTCAGGAGGTGGGGTGCTGTTAGAACAATGGTGTGCACCAGTGACACAA

      1930       1940       1950       1960       1970       1980
```

FIG. 3 (3/5)

AGATGTCATGGTTAAGATGGCATCAAGAAGTGGAAAGGACATTCGGAACAGTGGGTCCAA

1990 2000 2010 2020 2030 2040
GGCACCCAAAGTCCTCACCCCAATTTAGAAGCCGTTGGTCCTGTAAGACTTAAATCTACT

2050 2060 2070 2080 2090 2100
AAACAAGGAAGGTCTAACTGGGCTGGAATCTGAAGTTCATGGTGCCAGGCTGGGGCGGTG

2110 2120 2130 2140 2150 2160
GGTGGGGACGTGGCCGTGGCCATGACCATGATTGCCTCTCTGCATGGTGACACTTGCCTT

2170 2180 2190 2200 2210 2220
TTGCACCCTAGCTCTCAGCACATCTGAAAAGGACAGACTCTCCTGTTCATTCCTTGAATC

2230 2240 2250 2260 2270 2280
TGAGACTCTCCTCACTAATGTAGCAAAAATGGAGGTCTAAAGTGCAGGCTTCAGCCTCTG

2290 2300 2310 2320 2330 2340
AGGTCCAGGGCAGGAGGAAGCTGGGGCTCAGCCTCCTGGAGGATGAGAGCTTGCCGGGTG

2350 2360 2370 2380 2390 2400
AGCATCAGCGACAGCAGACCCTTGGGCTCAGAGAGTCCGCAAGCCTGGGAGAGCCTGGCC

2410 2420 2430 2440 2450 2460
GAGCCCTGACTGCAGCACACAGAGCCGTGAGCCTCATACAAGAAGCCACATTTTGGGGAA

2470 2480 2490 2500 2510 2520
GCTTCAGGGTGGCTGATTCCACAGCTGTTGGGTTCAGAACGGAAGCCGGGAGCACTCACT

2530 2540 2550 2560 2570 2580
TCAGATATGGAAGCTTTGTTTTACGAGCGCTTAGCACCAGTTCAGGATCTGAACTTCGTC

2590 2600 2610 2620 2630 2640
CTGACCGGAGAGTCCGTACCACATTTTTATAGGATGGGAACACAGAGCGAGGGGCGTGGA

2650 2660 2670 2680 2690 2700
GTAAGCTGTTGAACGACCGATCATATTTTGACCTAAGAGGTTAAGTAAGGACGTTAACAT

2710 2720 2730 2740 2750 2760
GGGTGACTGGGCATTAGTCAGGTCACCTGGTTTTGGGGTCTTTGAATCAGCTTTCGTGGC

2770 2780 2790 2800 2810 2820
CAGGTCCCTTCCTGGACTTTGGCTCGGAATTTAGAACGATAAGGGAACGAAGAGGTGGGC

2830 2840 2850 2860 2870 2880
AAGCTTCGGGCAGTCAGTAAGAGGCAGCACATTCATGACCTGTGTGCCTTGTTTAGATAA

2890 2900 2910 2920 2930 2940
TGGGATAAGAGTATCTCCTCTCTTACACCCCTTACTGGTTAACAGACAAACACGAGACAT

2950 2960 2970 2980 2990 3000
CTGAAGAAGCAGGACAGGAGTTAGGTTCTGGGGCACAGGAACATGAACTCGGTTTTGATC

3010 3020 3030 3040 3050 3060

FIG. 3 (4/5) 6/47

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CTGCCGGCAAGGTGGATCTTGTTCCTGAGAAGGCTGGACTCAGGAAACTTCCTCTTAACA

      3070       3080       3090       3100       3110       3120
AGTTAGTTGATGGCGCTGGTCCTTAGTCACCGATACTGTCAGGCTCTCAGCTCTTGGGCC

      3130       3140       3150       3160       3170       3180
AGACTTGGCGGCCATGGGAGTGTGGTCACTTGCCCCGTCCCCTTCTTCCAGGAGGTACTG

      3190       3200       3210       3220       3230       3240
GGGAAAATGGTTGGATTTGTGGAGTTGTAGGGAACACTCATGGCTCCCTTCACTTAGTAG

      3250       3260       3270       3280       3290       3300
GTCAGCTAACATATGTGTATCGAGCCCATACCGTGTGCCATGTGCAGTGCTGAGCAGCAG

      3310       3320       3330       3340       3350       3360
GGAGTCAGAGATTTAAAGACACACACACAGACTTCAAGTCTGAGAATTTTGAATCCCAGG

      3370       3380       3390       3400       3410       3420
GAGAACCGCTGAGAGCCATGGCGCTTCTACCAATGCCAGAGGCTAACACCCGGACTGAGA

      3430       3440       3450       3460       3470       3480
AAACTAAGCACGAGGAGACAGCAGGGTCAGCAGAGGGCCTGGGAGCTAGGGCCCTGAGCA

      3490       3500       3510       3520       3530       3540
GTACCTAGTTCAAATCACAGAGTCGTCTTTCTTCCTCCACCCTACCCAGGTACAGCAAGT

      3550       3560       3570       3580       3590       3600
AGACACGGGTGGGGGCAGGGCAGGGATGCAGGAACATTAGGGCACACCGATGTGGCTAGG

      3610       3620       3630       3640       3650       3660
CTAAGCTAGAGCATGTTACCTTCTCAGGGGTCCTGTCATGTCAGAGACTGGTTCCAACCT

      3670       3680       3690       3700       3710       3720
GGAAAGATGTCTGAGTGACAGCTGTGGTAGAAGAAGAGAGGCCAGGGTGATATCAGCATG

      3730       3740       3750       3760       3770       3780
AAGGGCTGGATTGCTATGTGAGATCCAGATCTCTTCTGCCACTGGGGTCAGCTTCTACAC

      3790       3800       3810       3820       3830       3840
TGGAAATAGATGGGCTGCGTTATGGAGGGTGGTGTGAGTCCCTGTCTGCGTTGTGCCGGG

      3850       3860       3870       3880       3890       3900
AATCAGAGCAGAGTGTTAGCGCTGTAAAAGGACATGCTGGTGCTTGCAGGAAATCATCGA

      3910       3920       3930       3940       3950       3960
TTTCTTGGAAGGGCAGCCATTCATCTACACCAGGGATTGACTTTATGCCAGGCTTGTGAT

      3970       3980       3990       4000       4010       4020
GAGGGTAGAAAAGTAGAAATTCTGTCCGCTGCAAGGAGCAGTCAGAGGACACAAGGACCA

      4030       4040       4050       4060       4070       4080
AATAGCTTGGGAGTTGCGGAAGTAGGTGTCTGCTGAGGGAGCAGTGACCACTGGGGGAAA

      4090       4100       4110       4120       4130       4140
```

FIG. 3 (5/5)

GGCTCCTTCAAGGAATTCAGGGACAGGGGTGAGGGCTGACATCTTGCCTGAGACCCTAAA

4150 4160 4170 4180 4190 4200
GAAGAGAAGGAGTTGAGAGGGCTGAGTATGCTGTGTGGAGCCCCACCCCCACCCCCACCC

4210 4220 4230 4240 4250 4260
CCACCCCCACCCCAGGTATATGGATGGAGGATAATGCGGGGGTCGGGTTCCTCTCAAATC

4270 4280 4290 4300 4310 4320
CATCATCCCACCTTCGAGCTGCTGGCACGGCCTTGCCAGCACAGCCCGATTCTGTGTTGA

4330 4340 4350 4360 4370 4380
CAAAATACTCGAACGAAATGATTACATGCAAATAAAATGCAAGAGGAAAAATCTAAACGG
Polyadenylation site

AATTC
clone linker

Fig. 4 RPLA2-8 Partial Genomic DNA and cDNA Structure

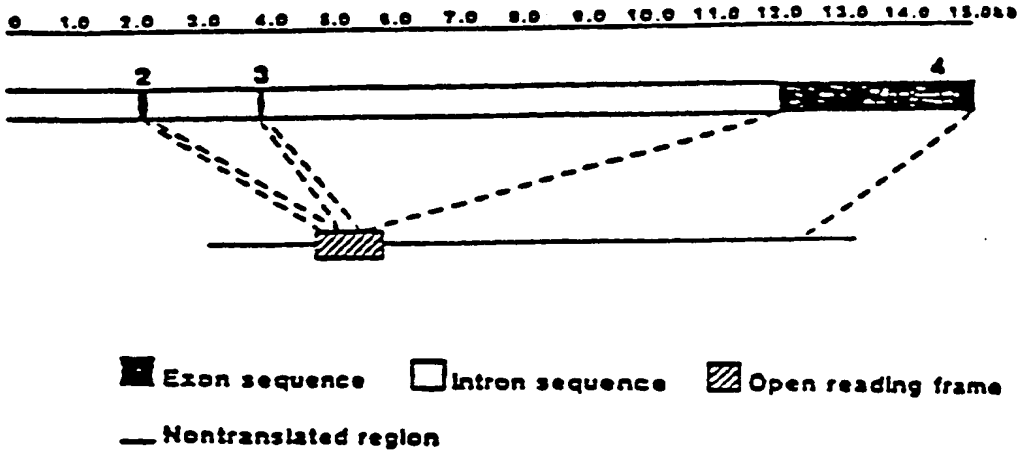

Fig. 5 Comparison Between HPLA2-8 Exon I and RPLA2-8 Exon I Sequences

```
400   ACCTCAGACCCCCTGGTCTCCTCAGGAATGAAGGTCATTGCCATCCTCACCCTCCTCCTC
      ||| |||| ||||||||||||||||||||||||| ||| || || || ||  || | ||
719   ACCATGGACCTCCTGGTCTCCTCAGGAATGAAGGGCATCGCTGTCTTCCTTGTCTTTATC

460   TTCTGCT
      |||||||
779   TTCTGCT
```

Matches = 51 Mismatches = 16 Unmatched = 0
Length = 67 Matches/length = 76.1 percent

Top strand is HPLA2-8 exon I sequence; bottom is RPLA2-8 exon I sequence.
The underlined ATG is the putative RPLA2-8 translation start codon.

Top strand is SEQ ID NO:23:;
Bottom strand is SEQ ID NO:24:.

Fig. 6 Comparison Between HPLA2-8 Exon II and RPLA2-8 Exon II Sequences

```
2633  tgg TGGCAGCCCCCACCCACAGCAGTTTCTGGCAGTTTCAGAGGAGGGTCAAACACATCACGG
          | || || |||||| |||||| |||||||||||| ||||||| ||||||||||||||||||
786   cag GGACAACCTCCACCCTCAGCAGCTTCTGGCAGTTCCAGAGGATGGTCAAACACATCACGG

2693      GGCGAAGTGCCTTCTTCTCATATTACGGATATGGCTGCTACTGTGGGCTTGGGGATAAAG
          |||| || |||||||||||| ||||||||||||||||||||||||||||||||||    ||
846       GGCGCAGCGCCTTCTTCTCCTATTACGGATATGGCTGCTACTGTGGGCTTGGGGGCCGAG

2753      GGATCCCCGTGGATGACACTGACAG gtg
          ||||||| ||||| | ||| |||||
906       GGATCCCTGTGGACGCCACAGACAG gtg
```

Matches = 126 Mismatches = 19 Unmatched = 0
Length = 145 Matches/length = 86.9 percent

Top strand is HPLA2-8 coding exon II sequence; bottom strand is RPLA2-8 exon II sequence

---

Top strand is SEQ ID NO:25:;
Bottom strand is SEQ ID NO:26:.

Fig. 7 Comparison Between HPLA2-8 Exon IV and RPLA2-8 Exon IV Sequences

```
13862  tag GTGGATGCACCCTTGGTCCTGGTGCCAGCTGCCACTGCAGGCTGAAGGCCTGTGAGTGT
           ||||||||||| || ||     ||||| |||||     ||| ||| ||| ||||||||||||
1034   cag GTGGATGCA CCATG       GGTGGCGGCTGCTTGTGCGGGCAGAAAGCCCTGTGAGTGT

13921      GACAAGCAATCCGTGCACTGCTTCAAGAGAGCCTGCCCACCTATGAGAAAAACTTCAAG
           ||||| |  || ||| |||||||||||| |||| |||||| |||||||| |||||| ||||||
1088       GACAAACTGTCTGTGTACTGCTTCAAGGAGAACCTGGCCACCTACGAGAAAACTTTCAAG

13981  CAG   TTCTCCAGCCGGCCCAGGTGTGGCAGACATAAGCCCTGGTGCTAG
       |||   ||| ||| | ||||| ||||||||| | || | |  ||||||
1148   CAGCTCTTCCCCACCAGGCCCCAGTGTGGCAGGGACAAACTCCATTGCTAG

Matches = 128     Mismatches = 33     Unmatched = 9
Length = 170      Matches/length =  75.3 percent

Top strand is SEQ ID NO:27:;
Bottom strand is SEQ ID NO:28:.
```

Fig. 8 Comparison of RPLA2-8 Deduced Amino Acid Sequence and Rat PLA2 Type I Amino Acid Sequence

```
1    MetAspLeuLeuValSerSerGlyMetLysGlyIleAlaValPheLeuValPheIlePhe
     |  |  |  |  :  :  :  :  :  :  |
1    MetLysLeuLeuLeuLeuAlaAlaLeu  LeuThrAla  GlyVal  Thr

21   CysTrpThrThrSerThrLeuSerSerPheTrpGlnPheGlnArgMetValLysHis
          :  |  |  :  |  |  |  |  |  :  |
16   AlaHisSerIleSerThrArgAlaVal  TrpGlnPheArgAsnMetIleLysCysThr

40   IleThrGlyArgSerAlaPhePheSerTyrTyrGlyTyrGlyCysTyrCysGlyLeuGly
     |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
35   IleProGlySerAspProLeuArgGluTyrAsnAsnTyrGlyCysTyrCysGlyLeuGly

60   GlyArgGlyIleProValAspAlaThrAspArgCysCysTrpAlaHisAspCysCysTyr
     |  |  |  |  |  |  |  |  |  |  |  |  :  |  |  |  |  |  |  |
55   GlySerGlyThrProValAspAspLeuAspArgCysCysGlnThrHisAspHisCysTyr

80   HisLysLeuLysGluTyrGly  CysGlnProIleLeu  AsnAlaTyr  Gln
     |  |  |  |  |  |  |  |  |  |  :  :  |  |  |  |  :
75   AsnGlnAlaLysLysLeuGluSerCysLysPheLeuIleAspAsnProTyrThrAsnThr

96   PheAla  IleValAsnGlyThrValThrCysGlyCysThrMetGlyGlyGlyCysLeu
      :  :  |  |  |  |  |  |  |  :
95   TyrSerTyrLysCysSerGlyAsnValIleThr  CysSerAspLysAsnAsnAsp

115  CysGlyGlnLysAlaCysGluCysAspLysLeuSerValTyrCysPheLysGluAsnLeu
     |  |  |  |  |  |  |  :  |  :  |  |  |  |  |  |  |  |  :
113  CysGluSerPheIleCysAsnCysAspArgGlnAlaAlaIleCysPhe  SerLysVal

135  AlaThrTyrGluLysThrPheLysGlnLeuPheProThrArgProGlnCysGlyArgAsp
        |  |  :  |  |  |  |  :
132  Pro  TyrAsnLysGluTyrLysAspLeu  AspThrLys

155  LysLeuHisCys
     |  |  |  |
144  Lys  HisCys
```

Matches = 56 Mismatches = 84 Unmatched = 24
Length = 164 Matches/length = 34.1 percent

Top line is RPLA2-8 deduced amino acid sequence; bottom line is rat type I PLA2 amino acid sequence. a vertical line indicates a match, : a conservative substitution, and no symbols a mismatch.

Top line is SEQ ID NO:22:;
Bottom line is SEQ ID NO:34:.

Fig.9 Comparison of the RPLA2-8 Deduced Amino Acid Sequence and Rat PLA2 Type II Amino Acid Sequence

```
1    MetAspLeuLeuValSerSerGlyMetLysGlyIleAlaValPheLeuValPheIlePhe
     |  :  |  :        :     :  | |   :  :      |
1    MetLysValLeuLeu     Leu   LeuAlaVal  ValIleMetAlaPhe

21   CysTrpThrThrSerThrLeuSerSerPheTrpGlnPheGlnArgMetValLysHisIle
          :           |           |       |  :
15   Gly  SerIleGlnValGlnGlySerLeuLeuGluPheGlyGlnMetIleLeuPheLys

41   ThrGly  ArgSerAlaPheSerTyr  TyrGlyTyrGlyCysTyrCysGlyLeu
     | |  |  :        | |   :   | | | |  | | :
34   ThrGlyLysArgAlaAspVal  SerTyrGlyPhe  TyrGlyCysHisCysGlyVal

59   GlyGlyArgGlyIleProValAspAlaThrAspArgCysCysTrpAlaHisAspCysCys
     | | | | |  |  | | | | |  | |  :  | | | |
52   GlyGlyArgGlySerProLysAspAlaThrAspTrpCysCysValThrHisAspCysCys

79   TyrHisLysLeuLysGluTyrGlyCysGlnProIleLeuAsnAlaTyrGlnPheAlaIle
     |  :  |     | |  |  |  :  |  |  :
72   TyrAsnArgLeuGluLysArgGlyCysGlyThrLysPheValThrTyrLysPheSerTyr

99   ValAsnGlyThrValThrCysGlyCysThrMetGlyGlyGlyCysLeuCysGlyGlnLys
        |  : :  |  |  |  |  |  |  |
92   ArgGlyGlyGlnIleSerCysSerThrAsn  GlnAspSerCysArg  LysGlnLeu

119  AlaCysGluCysAspLysLeuSerValTyrCysPheLysGluAsnLeuAlaThrTyrGlu
        |  |  |  :  |  |  |  |  :  |
110     CysGlnCysAspLysAlaAlaAlaGluCysPheAlaArgAsnLysLysSerTyrSer

139  LysThrPheLysGlnLeuProThrArgProGlnCys  GlyArgAspLysLeuHis
        :  |  :  |  :  |  |  |  :
129  LeuLysTyr  GlnPheTyrProAsnLys  PheCysLysGlyLysThrPro  Ser

158  Cys
     |
146  Cys
```

Matches = 56 Mismatches = 87 Unmatched = 18
Length = 161 Matches/length = 34.8 percent

Top line is RPLA2-8 deduced amino acid sequence; bottom line is rat type II amino acid sequence. 1 indicates match, : a consevative substitution and no symbol, a mismatch.

Top line is SEQ ID NO:22:;
Bottom line is SEQ ID NO:35:.

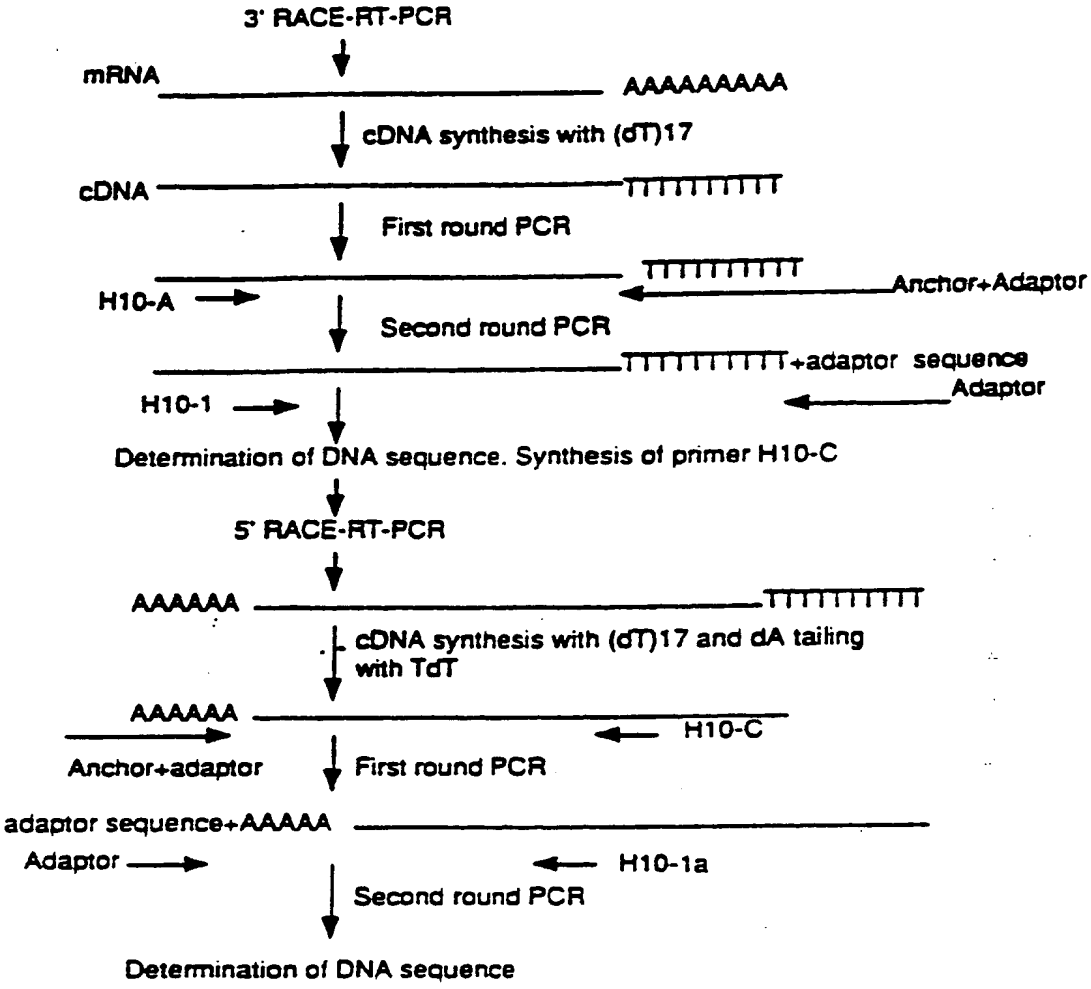

FIG. 10

Fig. 11 RPLA2-10 cDNA and Deriv d Amin Acid Sequence (1/3)

```
        10         20         30         40         50         60
GAATTCCGGTGGATGGAGGGGGCTGAGCAGGATGTTGACTGGCTATCGTTCATTGAGCAC
Clone linker
        70         80         90        100        110        120
TCTCACGATCAGCATCACGCACGGAATCCATCCTTCCTGTGTTGCAGCTTGTAGACCCTG

       130        140        150        160        170        180
ATGCTTGGGCTGCCAGCATAAACGTGGGGATCCAGACTCTGTCTACCGAGGCTGCCCATA
 gaattccggtccaggcctgtcctatgggcagcagcctcggtagacagagt....
 Clone linker
       190        200        210        220        230        240
GGGACAGGCCCTGGGAAGAGGAGCTGAGACCAGGCTAAAAAGAACCCAAGAAATGAAGCG
                                                    MetLysAr

       250        260        270        280        290        300
CCTCCTCACGCTGGCTTGGTTCCTGGCTTGCAGTGTGCCTGCAGTCCCAGGGGGCTTGCT
gLeuLeuThrLeuAlaTrpPheLeuAlaCysSerValProAlaValProGlyGlyLeuL
Rclo10-5' (primer)
       310        320        330        340        350        360
AGAACTGAAGTCCATGATTGAGAAGGTGACTGGGAAGAATGCCGTAAAGAACTATGGCTT
uGluLeuLysSerMetIleGluLysValThrGlyLysAsnAlaValLysAsnTyrGlyPh
Rclo10-1 (primer)
       370        380        390        400        410        420
CTACGGCTGCTACTGTGGCTGGGGCGGCCACGGGACCCCTAAGGATGGCACTGATTGGTG
eTyrGlyCysTyrCysGlyTrpGlyGlyHisGlyThrProLysAspGlyThrAspTrpCy
                                          Rclo10-2 (primer)
       430        440        450        460        470        480
CTGTCGGATGCACGACCGTTGTTATGGGCTACTGGAGGAGAAACACTGTGCCATCCGGAC
sCysArgMetHisAspArgCysTyrGlyLeuLeuGluGluLysHisCysAlaIleArgTh

       490        500        510        520        530        540
CCAGTCCTATGACTACAGATTCACACAGGACTTAGTCATCTGCGAACACGACTCCTTCTG
rGlnSerTyrAspTyrArgPheThrGlnAspLeuValIleCysGluHisAspSerPheCy

       550        560        570        580        590        600
TCCAGTGAGGCTTTGTGCTTGTGACCGGAAGCTGGTCTACTGCCTGAGGAGAAACCTCTG
sProValArgLeuCysAlaCysAspArgLysLeuValTyrCysLeuArgArgAsnLeuTr

       610        620        630        640        650        660
GAGTTACAACCGTCTTTACCAGTATTACCCCAACTTCCTCTGCTAATGTCCTCTGTGGGC
pSerTyrAsnArgLeuTyrGlnTyrTyrProAsnPheLeuCysEnd
                                  Rclo10-3' (primer)
       670        680        690        700        710        720
TCTCGCCGGGAGTGCCTCCCACAGTGGCGGCCCCCCTCGGCTGTATTCCTGATCCGTCCA

       730        740        750        760        770        780
CCCAAGGTCTTGGATCTGCCTTCCTCTGTGTACCACTGGGCTGGACAGAGCCCAGGGTTA

       790        800        810        820        830        840
CACCCTACCCTCCAGAATCCTAGAGAGGGACTCTGATGTAGAGTCTGCGGACTCTGGATA
```

RPLA 2-10 cDNA sequence corresponds to SEQ ID NO:29: and Derived Amino Acid sequence corresponds to SEQ ID NO:30:.

FIG. 11 (2/3) 16/47

```
        850         860         870         880         890         900
GCTGAGCCTGCACTTGCAGAATTTGGCGCTGGGCCCCGGAGCTCCCTCAGCTCCAGGCCA

910         920         930         940         950         960
GTGTCGTGTTGACTTTCCTTTCAATTTCTGGAACCCAACTGCCATTACCACCCTCCAGAG

        970         980         990        1000        1010        1020
ACCTCTTACTAGAGGAGAAGCCAAATTAACTCTATAAATCTGCCATGTAGCTATTAAATA

       1030        1040        1050        1060        1070        1080
AAACCCATTCACGAGGCGAGAAGAACACCACCCCAGCACTCCCTCTGACAGGGCTGGGGT

       1090        1100        1110        1120        1130        1140
AGGAGTGCCAATGCTTCTCTAACCCCTGAGGCATCTGTGCACCCTCTAGGATGGAGGTCA

       1150        1160        1170        1180        1190        1200
GGAAACAGGTGGGGGCCTTACATGCCTTTCATGGTTTGTCTTGAGTTTATTTTCTTAAAC

       1210        1220        1230        1240        1250        1260
CTTAGGGTCTTTCAAGCCAGACCTGGAGCTCAAGATTCTTCTGGAGGAAGGTGAGACACA

       1270        1280        1290        1300        1310        1320
GCCCTATGCCACCTTGAGCTCCAGGCTAGAAAGGGACAGCCCCTAGCCCTGGCTTCTGCA

       1330        1340        1350        1360        1370        1380
ACTGTGTGGTCTTGAACCTCCGTATAGTCCGAATCCCTCTGGCTCTCCTCAAAATATAAA

       1390        1400        1410        1420        1430        1440
ACAAGCCTCCTTCCAATAGCATATTGGTGCACACCCCTAATCCCATCACCTGGGAGGAGG

       1450        1460        1470        1480        1490        1500
AGGCGGCAGGAGCATCAGGAGTTCAAGGCCAGCTCCTGCCCCCTAGCAGGGATGGTAGGC

       1510        1520        1530        1540        1550        1560
TGCATGAGAGTGTGTCTCAGAAAGAACCACCTGGTGCGGGTACAGGGATGCTGGGATTCT

       1570        1580        1590        1600        1610        1620
GAGATGTCACTCAGTGCGGGAAAAGATTCAAGGAGGGGAACAGATCAATGGCAGAATGAC

       1630        1640        1650        1660        1670        1680
TGTCTGTGCCGAGTTAAGGGCACTGAAAATCTCAGCTCATCTATCGCTTTATAGAAGATA

       1690        1700        1710        1720        1730        1740
GAGCTTTGGGAGGAAGCAAGGCACTCTACAGTAAAGGAGTGGCCTTTCCAAGGAAGGGTC
                           Polyadenylation site
       1750        1760        1770        1780        1790        1800
TAGGCTCCTTCTTCTCCAGAACATGCACAGGACATAGGAGATCCATTATTTAGAGACCTT

       1810
TCGTGTTCGAACGTTTTCTCCGGAATTC----RPLA2-10-1
                    Clone linker
              ......aaataaagttaattatattgagccggaattc----RPLA2-10-2
   Additional Polyadenylation site.    Clone linker
```

FIG. 11 (3/3)

The top sequence comes from RPLA2-10-1. The bottom sequence is from RPLA2-10-2. Both the sequences are identical except for the 5' and 3' sequences indicated by the lower case letters.

Fig. 12 HPLA2-10 cDNA (Type IV) and Derived Amino Acid Sequenc

```
        10         20         30         40         50         60
GGATACCAATGTTCCGACTGGAGACGGGGAGCCCGCGAGACCCGGGTCTCCAGGGTCTGC
        70         80         90        100        110        120
CCAAGGAAGTTGCTCATGGGAGCAGACCCCTAGAGCAGGATTTGAGGCCAGGCCAAAGAG
       130        140        150        160        170        180
AACCCCAGAGATGAAAGGCCTCCTCCCACTGGCTTGGTTCCTGGCTTGTAGTGTGCCTGC
          MetLysGlyLeuLeuProLeuAlaTrpPheLeuAlaCysSerValProAl
   Hclo10-5'(primer)                Hclo10-A (primer)
   Clone HPLA2-10-5-----CCTCC....
       190        200        210        220        230        240
TGTGCAAGGAGGCTTGCTGGACCTAAAATCAATGATCGAGAAGGTGACAGGGAAGAACGC
aValGlnGlyGlyLeuLeuAspLeuLysSerMetIleGluLysValThrGlyLysAsnAl
      Clo10-1 (primer)                  Clone HPLA2-10-7----AACGC
       250        260        270        280        290        300
CCTGACAAACTACGGCTTCTACGGCTGTTACTGCGGCTGGGGCGGCCGAGGAACCCCCAA
aLeuThrAsnTyrGlyPheTyrGlyCysTyrCysGlyTrpGlyGlyArgGlyThrProLy
       310        320        330        340        350        360
GGATGGCACCGATTGGTGCTGTTGGGCGCATGACCACTGCTATGGGCGGCTGGAGGAGAA
sAspGlyThrAspTrpCysCysTrpAlaHisAspHisCysTyrGlyArgLeuGluGluLy
 Clo10-1a (primer)
       370        380        390        400        410        420
GGGCTGCAACATTCGCACACAGTCCTACAAATACAGATTCGCGTGGGGCGTGGTCACCTG
sGlyCysAsnIleArgThrGlnSerTyrLysTyrArgPheAlaTrpGlyValValThrCy
       430        440        450        460        470        480
CGAGCCCGGGCCCTTCTGCCATGTGAACCTCTGTGCCTGTGACCGGAAGCTCGTCTACTG
sGluProGlyProPheCysHisValAsnLeuCysAlaCysAspArgLysLeuValTyrCy
       490        500        510        520        530        540
CCTCAAGAGAAACCTACGGAGCTACAACCCACAGTACCAATACTTTCCCAACATCCTCTG
sLeuLysArgAsnLeuArgSerTyrAsnProGlnTyrGlnTyrPheProAsnIleLeuCy
                                                 Hclo10-C (primer)
       550        560        570        580        590        600
CTCCTAGGCCTCCCCAGCGAGCTCCTCCCAGACCAAGACTTTTGTTCTGTTTTTCTACAA
sSerEnd
Hclo10-3' (primer)
       610        620        630        640        650        660
CACAGAGTACTGACTCTGCCTGGTTCCTGAGAGAGGCTCCTAAGTCACAGACCTCAGTCT
       670        680        690        700        710        720
TTCTCGAAGCTTGGCGGACCCCCAGGGCCACACTGTACCCTCCAGCGAGTCCCAGGGGAG
       730        740        750        760        770        780
TGACTCTGGTCATAGGACTTGGTAGGGTCCCAGGGTCCCTAGGCCTCCACTTCTGAGGGC
       790        800        810        820        830        840
AGCCCCTCTGGTGCCAAGAGCTCTCCTCCAACTCAGGGTTGGCTGTGTCTCTTTTCTTCT
       850        860        870        880        890        900
CTGAAGACAGCGTCCTGGCTCCAGTTGGAACACTTTCCTGAGATGCACTTACTTCTCAGC
       910        920        930        940        950        960
TTCTGCGATCAGATTATCATCACCACCACCCTCCAGAGAATTTTACGCAAGAAGAGCCAA
       970        980        990       1000       1010
ATTGACTCTCTAAATCTGGTGTATGGGTATTAAATAAAATTCATTCTCAAGGCT
                    Polyadenylation site

                                                  .....AATAAA
                                                   Additional
AACCACATTGGCATTTTC----HPLA2-10-3
Polyadenylation site
```

HPLA2-10 cDNA sequence corresponds to SEQ ID NO:31: and Derived Amino Acid Sequence corresponds to SEQ ID NO:32:.

Fig. 13 **Comparison Between Deduced Amino Acid Sequences of HPLA2-10 and RPLA2-10**

```
1    MetLysGlyLeuLeuProLeuAlaTrpPheLeuAlaCysSerValProAlaValGlnGly
1    MetLysArgLeuLeuThrLeuAlaTrpPheLeuAlaCysSerValProAlaValProGly

21   GlyLeuLeuAspLeuLysSerMetIleGluLysValThrGlyLysAsnAlaLeuThrAsn
21   GlyLeuLeuGluLeuLysSerMetIleGluLysValThrGlyLysAsnAlaValLysAsn

41   TyrGlyPheTyrGlyCysTyrCysGlyTrpGlyGlyArgGlyThrProLysAspGlyThr
41   TyrGlyPheTyrGlyCysTyrCysGlyTrpGlyGlyHisGlyThrProLysAspGlyThr

61   AspTrpCysCysTrpAlaHisAspHisCysTyrGlyArgLeuGluGluLysGlyCysAsn
61   AspTrpCysCysArgMetHisAspArgCysTyrGlyLeuLeuGluGluLysHisCysAla

81   IleArgThrGlnSerTyrLysTyrArgPheAlaTrpGlyValValThrCysGluProGly
81   IleArgThrGlnSerTyrAspTyrArgPheThrGlnAspLeuValIleCysGluHisAsp

101  ProPheCysHisValAsnLeuCysAlaCysAspArgLysLeuValTyrCysLeuLysArg
101  SerPheCysProValArgLeuCysAlaCysAspArgLysLeuValTyrCysLeuArgArg

121  AsnLeuArgSerTyrAsnProGlnTyrGlnTyrPheProAsnIleLeuCysSer
121  AsnLeuTrpSerTyrAsnArgLeuTyrGlnTyrTyrProAsnPheLeuCys
```

Matches = 107 Mismatches = 30 Unmatched = 1
Length = 138 Matches/length = 77.5 percent

Top and bottom lines are deduced amino acid sequences of HPLA2-10 and RPLA2-10, respectively.

Top line is SEQ ID NO:32:;
Bottom line is SEQ ID NO:30:.

Fig. 14 Comparison Between HPLA2-10 Deduced Amino Acid Sequence and Human Type I Amino Acid Sequence

```
1         MetLysGlyLeuLeuProLeuAlaTrpPheLeuAlaCysSerValProAlaValGln
          |  |    |  |    |  |          |  :    :
1         MetLys   LeuLeuValLeuAlaValLeuLeuThrValAlaAlaAlaAspSerGlyIle

20        GlyGly   LeuLeu   AspLeuLysSerMetIleGlu   LysValThrGlyLysAsn
                   :        :     |  |            :     |
20        SerProArgAlaValTrpGlnPheArgLysMetIleLysCysValIleProGlySerAsp

37        AlaLeuThrAsnTyrGlyPheTyrGlyCysTyrCysGlyTrpGlyGlyArgGlyThrPro
               |        |  |  |  |  |  |     |  |     |  |  |
40        ProPheLeuGluTyrAsnAsnTyrGlyCysTyrCysGlyLeuGlyGlySerGlyThrPro

57        LysAspGlyThrAspTrpCysCysTrpAlaHisAspHisCysTyrGlyArgLeuGluGlu
          |     |        |  |  |  :  |  |     |  |
60        ValAspGluLeuAspLysCysCysGlnThrHisAspAsnCysTyrAspGlnAlaLysLys

77           LysGlyCysAsn   IleArgThrGlnSerTyrLysTyrArgPheAlaTrp
             |           :        :     |           :  :  :
80        LeuAspSerCysLysPheLeuLeuAspAsnProTyrThrHisThrTyrSerTyrSerCys

93           GlyVal   ValThrCysGluProGlyProPhe   CysHisValAsnLeuCysAla
             |        :  |  |                  |              :  |
100       SerGlySerAlaIleThrCysSerSerLysAsnLysGluCysGluAlaPheIleCysAsn

110       CysAspArgLysLeuValTyrCysLeuLysArgAsnLeuArgSerTyrAsnProGlnTyr
          |  |  |                 |        :              |  |
120       CysAspArgAsnAlaAlaIleCysPheSerLysAla      ProTyrAsnLysAlaHis

130       GlnTyrPheProAsnIleLeu   Cys   Ser
                                  |     |
138       LysAsnLeuAspThrLysLysTyrCysGlnSer
```

Matches = 45 Mismatches = 90 Unmatched = 16
Length = 151 Matches/length = 29.8 percent

Top line is HPLA2-10 deduced amino acid sequence; bottom line is human type I amino acid sequence.

Top line is SEQ ID NO:32:;
Bottom line is SEQ ID NO:36:.

Fig. 15 Comparison Between HPLA2-10 Deduced Amino Acid Sequence and Human PLA2 Type II Amino Acid Sequence

```
1    MetLysGlyLeuLeuProLeuAlaTrpPheLeuAlaCysSerValProAlaValGlnGly
     |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1    MetLysThrLeuLeuLeuLeuAlaValIleMetIlePheGlyLeuLeuGlnAlaHisGly

21   GlyLeuLeuAspLeuLysSerMetIleGluLysValThrGlyLysAsnAlaLeuThrAsn
     |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
21   AsnLeuValAsnPheHisArgMetIleLysLeuThrThrGlyLysGluAlaAlaLeuSer

41   TyrGlyPheTyrGlyCysTyrCysGlyTrpGlyGlyArgGlyThrProLysAspGlyThr
     |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
41   TyrGlyPheTyrGlyCysHisCysGlyValGlyGlyArgGlySerProLysAspAlaThr

61   AspTrpCysCysTrpAlaHisAspHisCysTyrGlyArgLeuGluGluLysGlyCysAsn
     |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
61   AspArgCysCysValThrHisAspCysCysTyrLysArgLeuGluLysArgGlyCysGly

81   IleArgThrGlnSerTyrLysTyrArgPheAlaTrpGlyVal   ValThrCysGluPro
     |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
81   ThrLysPheLeuSerTyrLysPheSerAsnSer   GlySerArgIleThrCysAlaLys

100  GlyProPheCysHisValAsnLeuCysAlaCysAspArgLysLeuValTyrCysLeuLys
     |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
100  GlnAspSerCysArgSerGlnLeuCysGluCysAspLysAlaAlaAlaThrCysPheAla

120  ArgAsnLeuArgSerTyrAsnProGlnTyrGlnTyrProProAsnIleLeuCys   Ser
     |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
120  ArgAsnLysThrThrTyrAsnLysLysTyrGlnTyrTyrSerAsnLysHisCysArgGly

140  SerThrProArgCys
```

Matches = 63 Mismatches = 74 Unmatched = 8
Length = 145 Matches/length = 43.4 percent

Top line is HPLA2-10 deduced amino acid sequence; bottom line is human PLA2 type II amino acid sequence.

Top line is SEQ ID NO:32:;
Bottom line is SEQ ID NO:37:.

Fig. 16 . Comparison Between Deduced Amino Acid Sequences of RPLA2-10 and Rat PLA2 Type II Amino Acid Sequence

```
1         MetLysArgLeuLeuThrLeuAlaTrpPheLeuAlaCys   SerValProAlaValPro
          |  |  |  |  |  |  |  |  |  |  |  :  |  |  |  |  |  |  |  |
1         MetLysValLeuLeuLeuLeuAlaValValIleMetAlaPheGlySerIleGlnValGln

20        GlyGlyLeuLeuGluLeuLysSerMetIleGluLysValThrGlyLysAsnAlaValLys
          |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
21        GlySerLeuLeuGluPheGlyGlnMetIleLeuPheLysThrGlyLysArgAlaAspVal

40        AsnTyrGlyPheTyrGlyCysTyrCysGlyTrpGlyGlyHisGlyThrProLysAspGly
          |  |  |  |  |  |  |  |  |  |  |  |  :  |  :  |  |  |  |  |
41        SerTyrGlyPheTyrGlyCysHisCysGlyValGlyGlyArgGlySerProLysAspAla

60        ThrAspTrpCysCysArgMetHisAspArgCysTyrGlyLeuLeuGluGluLysHisCys
          |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  :  |  |  |
61        ThrAspTrpCysCysValThrHisAspCysCysTyrAsnArgLeuGluLysArgGlyCys

80        AlaIleArgThrGlnSerTyrAspTyrArgPheThrGlnAspLeuValIleCysGlu
                :  |  :  |  :  |  :  |  |  :  |  |  :  |  |  |  |
81        GlyThrLysPheValThrTyrLysPheSerTyrArgGlyGlyGlnIleSerCysSerThr

99        HisAspSerPheCysProValArgLeuCysAlaCysAspArgLysLeuValTyrCysLeu
          |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
101       AsnGlnAspSerCysArgLysGlnLeuCysGlnCysAspLysAlaAlaAlaGluCysPhe

119       ArgArgAsnLeuTrpSerTyrAsnArgLeuTyrGlnTyrTyrProAsn   Phe
          |  |  |  |  |  |  |  |  |  |  |  |  :  |  |  |     |
121       AlaArgAsnLysLysSerTyrSerLeuLysTyrGlnPheTyrProAsnLysPheCysLys

136             Leu       Cys
                |         |
141       GlyLysThrProSerCys
```

Matches = 62 Mismatches = 75 Unmatched = 9
Length = 146 Matches/length = 42.5 percent

Top line is RPLA2-10 deduced amino acid sequence; bottom line is rat PLA2 type II amino acid sequence.

Top line is SEQ ID NO:30:;
Bottom line is SEQ ID NO:35:.

Fig. 17. Comparison Between Deduced Amino Acid Sequences of RPLA2-10 and RPLA2-8

```
1    MetLysArgLeuLeuThr      Leu  Ala          Trp       PheLeuAla
1    MetAspLeuLeuValSerSerGlyMetLysGlyIleAlaValPheLeuValPheIlePhe

13   Cys  SerValProAlaValProGlyGlyLeuLeuGluLeuLysSerMetIleGluLys
21   CysTrpThrThrSerThrLeu  SerSerPheTrpGlnPheGlnArgMetValLysHis

32   ValThrGlyLysAsnAlaValLysAsnTyrGlyPhe  TyrGlyCysTyrCysGlyTrp
40   IleThrGlyArgSerAlaPhePheSerTyr  TyrGlyTyrGlyCysTyrCysGlyLeu

51   GlyGlyHisGlyThrProLysAspGlyThrAspTrpCysCysArgMetHisAspArgCys
59   GlyGlyArgGlyIleProValAspAlaThrAspArgCysCysTrpAlaHisAspCysCys

71   TyrGlyLeuLeuGluGluLysHisCysAlaIleArgThrGlnSerTyrAspTyrArgPhe
79   TyrHisLysLeuLysGluTyrGlyCysGlnProIleLeuAsnAlaTyrGlnPheAlaIle

91   ThrGlnAspLeuValIleCysGlu  His  AspSer      PheCysProValArg
99   ValAsnGlyThrValThrCysGlyCysThrMetGlyGlyGlyCysLeuCysGlyGlnLys

107  LeuCysAlaCysAspArgLysLeuValTyrCysLeuArgArgAsnLeuTrpSerTyrAsn
119  AlaCysGluCysAspLysLeuSerValTyrCysPheLysGluAsnLeuAlaThrTyrGlu

127  ArgLeuTyr  GlnTyrTyrProAsnPhe                    Leu  Cys
139  LysThrPheLysGlnLeuPheProThrArgProGlnCysGlyArgAspLysLeuHisCys
```

Matches = 48 Mismatches = 87 Unmatched = 25
Length = 160 Matches/length = 30.0 percent

Top line is RPLA2-10 deduced amino acid sequence; bottom line is RPLA2-8 deduced amino acid sequence.

Top line is SEQ ID NO:30:;
Bottom line is SEQ ID NO:22:.

Fig. 18 Comparison Between Deduced Amino Acid Sequence of RPLA2-10 and Rat PLA2 Type I Amino Acid Sequence

```
1         MetLysArgLeuLeuThrLeuAlaTrpPheLeuAlaCysSerVal   Pro   AlaVal
1         MetLys   LeuLeuLeuLeuAlaAlaLeuLeuThrAlaGlyValThrAlaHisSerIle

19        ProGly   GlyLeuLeuGluLeuLysSerMetIleGlu   LysValThrGlyLysAsn
20        SerThrArgAlaValTrpGlnPheArgAsnMetIleLysCysThrIleProGlySerAsp

37        AlaValLysAsnTyrGlyPheTyrGlyCysTyrCysGlyTrpGlyGlyHisGlyThrPro
40        ProLeuArgGluTyrAsnAsnTyrGlyCysTyrCysGlyLeuGlyGlySerGlyThrPro

57        LysAspGlyThrAspTrpCysCysArgMetHisAspArgCysTyrGlyLeuLeuGluGlu
60        ValAspAspLeuAspArgCysCysGlnThrHisAspHisCysTyrAsnGlnAlaLysLys

77           LysHisCysAla   IleArgThrGlnSerTyr   Asp   TyrArgPhe
80        LeuGluSerCysLysPheLeuIleAspAsnProTyrThrAsnThrTyrSerTyrLysCys

91        ThrGlnAspLeuValIleCys   GluHisAspSerPheCysProValArgLeuCysAla
100       SerGlyAsnValIleThrCysSerAspLysAsnAsnAspCysGluSerPheIleCysAsn

110       CysAspArgLysLeuValTyrCysLeuArgArgAsnLeuTrpSerTyrAsnArgLeuTyr
120       CysAspArgGlnAlaAlaIleCysPheSerLys   Val   ProTyrAsnLysGluTyr

130          GlnTyrTyrProAsnPheLeuCys
138       LysAspLeuAspThrLysLysHisCys
```

Matches = 45 Mismatches = 89 Unmatched = 15
Length = 149 Matches/length = 30.2 percent

Top line is RPLA2-10 deduced amino acid sequence; bottom line is rat PLA2 type I amino acid sequence.

Top line is SEQ ID NO:30:;
Bottom line is SEQ ID NO:34:.

Fig. 19 Human Genomic HPLA$_2$-8 Sequence
(1/15)

SEQ ID NO:33:

```
        10          20          30          40          50          60
AAGCTTTGTG GGATTTCTAT TATGAACAAC ATAGGTGCCT TTCCAACTCG GGAACAGAGG

        70          80          90         100         110         120
AAATATGGAC TCCTCAAAAG AAAAAAAGAA GAGATGAAGG GATGATGTTG CCAAAGAAAG

       130         140         150         160         170         180
AAATTTGGAA AAAAAAAAAC CAAACCAACA TTTGCACTTT CAAAACCATG GAACCCTTCT

       190         200         210         220         230         240
TATTTTTATA TGTTCAGATC TAAATGCCAG AAAGGTTACC ACATTCAAAG GGAATGAGAT

       250         260         270         280         290         300
TTGAAAATGA TTTCTTTGAG TCCTCTGCTG AGGTCTTTCC AAGGCACTAC AATTAGGGCT

       310         320         330         340         350         360
TTGCACCCAA ATACCCTTGC CTCATTTTGG TCATTTTTGT CCTGGAACAG AGGTTCAGCT

       370         380         390         400         410         420
GGGAGACCCC TCACACACAG GTGAAGGCGT GGCTGTAGAA CCTCAGACCC CCTGGTCTCC
                                                    Exon 1 ?

       430         440         450         460         470         480
TCAGGAATGA AGGTCATTGC CATCCTCACC CTCCTCCTCT TCTGCTGTAA GTAGAGAGCG

       490         500         510         520         530         540
TTGGTGGGTC AGCACCAAGC TTCTGTCTTC CTGTTTATGT CAGTGGGAGG GGGGACTCTC

       550         560         570         580         590         600
CAGGTGGCAC CAGGTGAGGG AAGTCACAAG TCCCGCAGAA AAGAATCAGG AAAGGAACGG

       610         620         630         640         650         660
GCTCCCACCA ACGTCCTCTT GCTTCTGTTT CTGCTATAAA ATGGGCTGAT CCCAGTGTTG

       670         680         690         700         710         720
GGATCTTATA AAGTGTCTAG GAAATCAGAG GTTGCCAACC ATTTGCTAGA AAGGGAGTTT

       730         740         750         760         770         780
GAGTAGTATT TTACCCCCCC TCACCCTCAA GAGTCTTTTT ACTTTGGATG CTAGTAGCCT

       790         800         810         820         830         840
TTTATTTAGG CATTGGATCA GAACAAAAAT GCAGGACATA TATCCAGCCT AATTTAACCA

       850         860         870         880         890         900
ATGGATTAAA TGGCCTTATC AGGAAAAGAC CATTTTATGG TGACTTATGG GATAATTGGT

       910         920         930         940         950         960
AGTTATAAGT CATTGCTGCC GGGAGATCCG ATTGCTTACC TCTGCAAAGT GAAGAAAGAC

       970         980         990        1000        1010        1020
CTACTGGGAA ACAGTTTGGG GTCTACTGGA GACTGATAGA CTCTTTTGCT GGATTCGTTG
```

FIG. 19 (2/15)

```
      1030        1040        1050        1060        1070        1080
AGTGGAGGTT TCTCCAGATC CATTTTCCTG TCTCTTTCAA TTGAGTCACA ATAACTTTTG

      1090        1100        1110        1120        1130        1140
AGTCCCTAAG TCAAAGATGT CAAAAACAGA CTTCCTTTCC CCACAGTGAG TGGTGGAATT

      1150        1160        1170        1180        1190        1200
TACACTTTGC AAGGTGATAG TGCAGGAGGA TACCTGTACG CAGGGATGAC CGCCTCTGCA

      1210        1220        1230        1240        1250        1260
GCCCTCAGTG CGGCTCCAGG ACTGCTTGGG CACCAGTGAC CGCCCCATGG GTTTCTTCCG


      1270        1280        1290        1300        1310        1320
CCACACCCCC GTTTAGACTG AACACGATAG GTAGATCGAA GGCCACCTGA GAAAACTCCC

      1330        1340        1350        1360        1370        1380
CCAAAACTCT ATTTCTGTTT CTCTTCTTCA AAGTTCATGT CTTTGTTGTA TTTTTATTGC

      1390        1400        1410        1420        1430        1440
AAATTTACTA CATGCTTATA GTTAAAAAGT AAAATAAATG AGTATATAGC AACAAGGTAA

      1450        1460        1470        1480        1490        1500
AGCTCCTCCT CATCCTCCCC AGACCCCAGT TTTTTCCCTA CATCCAGATG TGACCACTCT

      1510        1520        1530        1540        1550        1560
TAAGAGTTTG ATATACATCC TCTATACAGC GTTTACCACA CACACATTCA AAACACCATA

      1570        1580        1590        1600        1610        1620
ATAGGAAGGG AACACATGCT GGGCCGGGCG CGGTTGTTCA TGACTATAAT CCCAGCACTT

      1630        1640        1650        1660        1670        1680
TGGGAGGCCG AGGCGGGCGG ATCACCTGAG GTCAGGAGTT CGAGACCAGC CTGGCCAGCT

      1690        1700        1710        1720        1730        1740
GGCAACATGG TGAAACCCGT CTCTATTAAA AATACAAAAA ATTAGTCAAG CATGGCAGTT

      1750        1760        1770        1780        1790        1800
GGGCACCTGT AATCCCAGCT ACTCAGGAGG CTGAGGCAGG AGAATTGCCT GAACCCGGGA

      1810        1820        1830        1840        1850        1860
GGCGGAGGTT GCAGTGAGCC GAGATCACAC CATTGCACTC CAGCCTGGGT AACAACAGCG

      1870        1880        1890        1900        1910        1920
AAACTCCGTC TCAAAAAAAA AAAAAAAAGA AGGAAAGGGA CACACGCTTA TTATGAAAGA

      1930        1940        1950        1960        1970        1980
CATGAGACAG CGGAGACGTG TATAAATGAT GTTGCCTGTT TTCTTTCTCT CTCTTCATCC

      1990        2000        2010        2020        2030        2040
ATGCTAGAGA TAGTGCTATC AAATGTAGTT ATTTTTGAGA CACATATTTC GTATTATCCC

      2050        2060        2070        2080        2090        2100
TGTCGTGACA TGTGGGTGGT TTCCAATTTT TTGATATCAC AGATAATGCT TCAGGAAACC
```

FIG. 19 (3/15)

```
      2110        2120        2130        2140        2150        2160
ATTTTGTGTA TCGATTTGTG CCCACTCTCA TAAGCATCTT GTAGAAGCAA AAACAGCTGA

      2170        2180        2190        2200        2210        2220
GTTCATGTGT ACTTGTCATT TAAAAAAATA ATAATTGAGG ATACCTTTCC TGCCTCTTAA

      2230        2240        2250        2260        2270        2280
GTATTTTGTT TCTCCTGTGA GATAGTAAAG GCCTGATGAC ATCTGGAGGG ACTGGCGTTT

      2290        2300        2310        2320        2330        2340
CTGGCTTTGA ACTTTTGCCA TTCATGTTGC ATCAGACCCG AGGGTGTTCT GCCTAGAACT

      2350        2360        2370        2380        2390        2400
GTGGTTTCTT GCTTTGAGGG GGAAGACTAT GGTTGATGGG AAAGCCTTGT TCTGAACCTC

      2410        2420        2430        2440        2450        2460
ATGGAAACTG GGTATTCATC TGGGTTAGCA AAAAACTAGC TGTGTTACAG GGGCAAATCT

      2470        2480        2490        2500        2510        2520
GAACCTATTT TATTCCCCAG GAAAGAGGCT GGTGATTCCA GCCATGCCCC TTGCACTTCG

      2530        2540        2550        2560        2570        2580
CTTTGGGGAT CTGGTGATAT TTCGAATGCT CAGCACTCTA GTAAGGGGAG GGGACATCAA

      2590        2600        2610        2620        2630        2640
GGCAGCATCA TGCTCATTGC AACTTCCTTC TTCCTTTTTT TCTCATCGGT GGTGGCAGCC

      2650        2660        2670        2680        2690        2700
CCCACCCACA GCAGTTTCTG GCAGTTTCAG AGGAGGGTCA AACACATCAC GGGGCGAAGT

      2710        2720        2730        2740        2750        2760
GCCTTCTTCT CATATTACGG ATATGGCTGC TACTGTGGGC TTGGGGATAA AGGGATCCCC
 Exon 2

      2770        2780        2790        2800        2810        2820
GTGGATGACA CTGACAGGTG GGTGCAGAGG CTCTAAGGCC ACTTATCATT TGTTTTGCAT

      2830        2840        2850        2860        2870        2880
TAAAGTTCAT GCTCAAAGCC AGAGAGAGGG TCTTAGGATT CTTGCCTGGC AAATAACAGA

      2890        2900        2910        2920        2930        2940
AAACAACTCA GGCTAATGGA AGGAAGAACT GAACGGGATT TGGAGGATGG GTCTTGAGAA

      2950        2960        2970        2980        2990        3000
ACCCAGGGTC GGGGCCAGCT TCTTGAGTGT GTGACCTGTG AAGTTTCACA GGGCCCAACA

      3010        3020        3030        3040        3050        3060
CTCATAAGGG TCAGGGCCAG CTTCTTGAGC GTGTGATCTG TAAAGTTTCA CAGGGCCTGG

      3070        3080        3090        3100        3110        3120
CACTCATAAC CCCCTAAACA TGGTTTACTG CTCTGCTGCC ACATCTTGAA ATTCTTAATA
```

FIG. 19 (4/15)

```
      3130        3140        3150        3160        3170        3180
AAGGGCCTCA TGTTTTCATT TTGCTTTACT CTCTGCAATT ATGCCGTTGG TCCTGCCCAG

      3190        3200        3210        3220        3230        3240
AGCTCTAGAA GCTGTTTCAT CCTCATAGTA AAAGTGCTCT GCTTTCAGCT CTCCAGCTTT

      3250        3260        3270        3280        3290        3300
TAGCACTATA CCCACAGCAC AACTGACTCA CTAGTCCTAA TTCCATATTC TGGAGAGGGC

      3310        3320        3330        3340        3350        3360
TCCAAAGTGG CCCACTTTGG AGAAGTTGTC CATCTGGGTG AGGTTGCATG GCACAAACCT

      3370        3380        3390        3400        3410        3420
GGCTTCAGGC CTACTCCAAA GGATGGGGGT GGGGGAGTGT GAGTTCCTAG AAAAAGTAGA

      3430        3440        3450        3460        3470        3480
GGTGGGTGTC ATCTGGTGAA TGTACGTGTG GGGAGGTAAG AAACGGGACA GTTTGCGTCT

      3490        3500        3510        3520        3530        3540
CAATTCATTT GAAGACATAA GAAAGCAAAA TGTTCCTTGC CACATTTAAG GTAGTATGGA

      3550        3560        3570        3580        3590        3600
GAAACATGTC CCACAGTGGC CTTAAATATC ACTCTGAGCT CGAGTCTTGT GGTGGCTCAT

      3610        3620        3630        3640        3650        3660
GAACCATGGA GGACCTAGAG GTTCGAAGGG CAATTGACGC TTATCAAATG CCCTTATGTG

      3670        3680        3690        3700        3710        3720
CCAAGCACTG GGACTGGCCG ATTGGCATAC AAACCTAATT TAATTCTCGC AGGGAATGCA

      3730        3740        3750        3760        3770        3780
CGACACAGTT GATACCAGCC CATTTGACAG CCTGAGGACA TGTGAGTTGC TAAACCACCT


      3790        3800        3810        3820        3830        3840
CCTAAAGGCA ATGCAGCTTC TAAGTGGCAG AGTTTAGGAT TGAACGAGAA TTTGCCTATT

      3850        3860        3870        3880        3890        3900
TCAAAGTTTG TCCCCTCTCC TTGATGGTCT GTGCCTCCCC TGTCAAAGTC CAAAGGCTGA

      3910        3920        3930        3940        3950        3960
TTAGAAATTG AACATCATTA GCCAAAGCTG ATCAACAGCA GAGCCCCCAC TTGCAGATGG

      3970        3980        3990        4000        4010        4020
GAATGGTGAG AGAGGGAGAC TGAAACACTT TTTTCTTGGC CTTTCAGGGT TTAGAATCCA

      4030        4040        4050        4060        4070        4080
AGCTTAAGTT TCTGCCTTCC TGTCCCTTGT GTAGTGGTTG AGGACATGGA CTGAGCCCAT

      4090        4100        4110        4120        4130        4140
GCTCCAGATG GTATTTCTCC TCCAGTGCTC TCCCATCCAG CCCCCAGCCA ACTCTGGGTG

      4150        4160        4170        4180        4190        4200
CCATGAATGG GACTACGTCG GCTTTTACAG ACAGTTGTCT CCTCAGAGAC CGTTACAGTG
```

FIG. 19 (5/15)

```
      4210        4220        4230        4240        4250        4260
CCTGACTCAC AGTAGGTGCT CAGTAAAAAG TGTTAAATGA ATGAATGGGC CTAGGTTTGT

      4270        4280        4290        4300        4310        4320
GTCCTGGGTC TATCATTCTC CAGCTGCCTA AGTTTGGGAA ATTGGCCTCT TGGAATCTCA

      4330        4340        4350        4360        4370        4380
GTCCCTCCCC TACAAAAGGG CAGCAATGAT TGTACTTTAT AGTTTCTAGT AGCTAATGAG

      4390        4400        4410        4420        4430        4440
ATAGCAACAG ATACTACAGA GGGCTCAGGA AATGCTACTG GTTATTATTA TTATTTTTTA

      4450        4460        4470        4480        4490        4500
TTTTATTTAT TTTTTGGGAG ACGGGGTCTT GCTCTATTAT CCAGGCCTGG GGTGGAGAGG

      4510        4520        4530        4540        4550        4560
CTCAATCAGA GCTCACTGCA GGTCCTCAAG CAATCCACCC ACTTCACCTC CTGAGTAGCC

      4570        4580        4590        4600        4610        4620
GGGACCACAG GCTGGTGCCA CCATGCCTGG CTTTTTTTTT TTTTTTAAAC TTAAAAAACA

      4630        4640        4650        4660        4670        4680
TAGGCGGCTC CCTATGTTGC CCAGGCTGGT CTCAAACTCC TGGACTGAAG CGATCCTCCT

      4690        4700        4710        4720        4730        4740
GCCTTATCCT CACAAAGTGC TGGGATTGCA GGCATGAGCC ACCACACCTG GCCTATGTTT

      4750        4760        4770        4780        4790        4800
AATATTATTG ATAATTCACC TCCTCACCTT CAATGCCTTC TTGCCTAGAG GAGGAGGCAG

      4810        4820        4830        4840        4850        4860
GTGAGCCCTT TCTAGTCCCC AGATAAGGTC CTCCAGCAGA TTCCTGAGGG ACCCACTTCC

      4870        4880        4890        4900        4910        4920
AGGCACAGCC CCTCATCTCC CTCTCCCTAC GAGAAGCTGA AGGAGTTCAG CTGCCAGCCT

      4930        4940        4950        4960        4970        4980
GTGTTGAACA GCTACCAGTT CCACATCGTC AATGGCGCAG TGGTTTGTGA GTAGCCTTTT

      4990        5000        5010        5020        5030        5040
CTGTATGGAA ATGTCTTTTA ACCTGGGCCT TTCCTTAACG TTCACCTCCT CTTTGACCCA

      5050        5060        5070        5080        5090        5100
GAGATCTTTT AGAAAATGAA ATGCTTCCAA GTGCTTGGAA GGAGATATTC CTGAGCTTTC

      5110        5120        5130        5140        5150        5160
TCCTGATGCT CCAGAGCTTC TCAGAGTGTC CGTGCTCATC CTGCCCTGGT CTCTCCCACC

      5170        5180        5190        5200        5210        5220
CATGAGTGTA CCTCCTGAAC TCTCTGGGGG CCCAGAGCCT GGCAGATAGT ACATGCTCAG

      5230        5240        5250        5260        5270        5280
TAAATACTTG TTCACTTGAG CTAATCTTGA AGCTTCCCTT GACAACTGCT GCTGTTGAGA
```

FIG. 19 (6/15)


```
      5290       5300       5310       5320       5330       5340
ACATGTTTCC TTGTTTCTGT GATTTTGTTA ACAAAACGGC TCAGCTGTCT TCCAGTTGGA

      5350       5360       5370       5380       5390       5400
CAAATATTTA TTAAGGGCGA CTGCATGCCA AGCACTAAGA TAGGTGCTGC CAGGGCCACA

      5410       5420       5430       5440       5450       5460
AAAGCAAATA GGTGGGAAGG GAAGGGGGAC TCACATGTTA CTGAGACCAT TCAAGGAGCC

      5470       5480       5490       5500       5510       5520
ATGTGGGCAA GTGGATCAGT GCCCTTCACA TGGGGCGTGG CCTGGCATCC GGAGCGTGTT

      5530       5540       5550       5560       5570       5580
CTGCGGCTGG TAGGGTATGG GTATGTGCAG GGCAATCCTG GCCTAGACAG CAGGCACATT

      5590       5600       5610       5620       5630       5640
TGGAGGCACG GGACAGTAGT CTTTCGTGAG CACCATCCTT TCCAGCATAG CCAGGGTGGA

      5650       5660       5670       5680       5690       5700
TCCTGGGGTC CTGGGCTGGG AGGGTGAAGA GCAACAAATA AAGAAGTGGC TTCTTGGCCG

      5710       5720       5730       5740       5750       5760
GGCGCGGTGG CTCACGCTTG TAATCCCAGC ACTTTGGGAG GCCGAGGCGG GCGGATCACG

      5770       5780       5790       5800       5810       5820
AGGTCAGGAG ATCGAGACCA TCCTGGCTAA CACGGTGAAA CCCCGTCTCT ACTAAAAATA

      5830       5840       5850       5860       5870       5880
CAAAAAAAAT TAGCCGGGCG TGATGGTGGG CGCCTGTAGT CCCAGCTACT CGGGAGGCTG

      5890       5900       5910       5920       5930       5940
AGGCAGGAGA ATGGCGTGAA CCCGGGAGGC GGAGCTTGCA GTGAGCCGAG ATTGCGCCAC

      5950       5960       5970       5980       5990       6000
TGCACTCCCG CCTGGGCCAC AGAGCGAGAC TCCGTCTCAA AAAAAAAAAA AAAAAAAAAG

      6010       6020       6030       6040       6050       6060
AAGAAGTGGC TTCTTATAGT GTGTGGCTCA CTTCCTGCCT GGCCTCGTGG GGTTGCATGA

      6070       6080       6090       6100       6110       6120
ATCACTTTCC TTCCCAGGTG TATTTATTCA GAGCTGTGAG TGCACCTTGG AGTTCCTCTG

      6130       6140       6150       6160       6170       6180
TTTCCTCCTG AGGTCAGGGA ACTACCACCT CTCTGCCACT CATCCCCTAT GGCGGGAGAT

      6190       6200       6210       6220       6230       6240
ACATCCTCCA TCCCGTAGTG GGTTCCAGGG CTCAGAACCC TGGTACTCCT GAGCTCCCCA

      6250       6260       6270       6280       6290       6300
ACCCACCACT TCAGCTCAGC ACACACCAAT ACCCAGAGTT AGGACTGTGA GGTCTCCCTG


      6310       6320       6330       6340       6350       6360
GCACCAGCTG TGTGGGTTGG GGGCTCGGAC CCCTGCACCG GGAGGACCTG CCTCAGCTCT
```

FIG. 19 (7/15)


```
      6370       6380       6390       6400       6410       6420
TGGCCTGCCC TGCCCACTGC CACCAGCACG TGGTTGACAG GGAAAGAACC CCCTTTTGTT

      6430       6440       6450       6460       6470       6480
CCCCACGTGA GCTCAAGCAA TCCACCCACT TCAGCCTCCT GAGTAGCTGG GATTACAGGT

      6490       6500       6510       6520       6530       6540
GCCCACTGCC ATGCTTGACT AATTTTTTGT ATTTTTAATA GAGACGGGGT TTCACCATCT

      6550       6560       6570       6580       6590       6600
TGGCCAGCTC AGCACACACC AATACCCAGA GTTAGGACTG TGAGGTCTCC CTGGCACCAG

      6610       6620       6630       6640       6650       6660
CTGTGTGGGT TGGGGGCTCG GACCCTGCAC CGGGAGACCT GCCTCAGCTC TTGGACTGCC

      6670       6680       6690       6700       6710       6720
TGCCACTGCC ACCAGCACGT GTTGACAGGG AAAGAACCCC TTTTGTTCCC ACGTGAGCTC

      6730       6740       6750       6760       6770       6780
AAGGAGACTT CCCTGAGTTG GAGCTCTCTG GTGTGGTCCT TCTCAGGCCT AAAGCAAAGT

      6790       6800       6810       6820       6830       6840
GTCTTTTCTG TGACACCTCC AAGGCCATGT TCAGGAGAGG GGAAGGGATC AGGGCCTGGT

      6850       6860       6870       6880       6890       6900
GGGAGGGATG GGGAGAGGGG ACTGGAGAAG GTGGCCTCCA GGGATCGAGT TTCCCATGGC

      6910       6920       6930       6940       6950       6960
CTCTTCCCAC CTGTCTTTGC CACAGGGGTG GGGACACCTG GCTGGCCCAG CCCAAGCCTC

      6970       6980       6990       7000       7010       7020
CACCCTGGGC TCCTGTGGGC TGGCTGCACT CGCCAGGGCT GGCCTAGGCT CTCTGCACCC

      7030       7040       7050       7060       7070       7080
AGGGAAGCTT CTCTATTCAA TGCTCTTCAC CCTCCCAGCC CAGGACCCCA GGAGATGAGG

      7090       7100       7110       7120       7130       7140
GAGAGTGGAG CAAAGGTTGA GGAGCAGAGG CTGGAGCCCC AGGCAGTGGC ACTGCTGGGC

      7150       7160       7170       7180       7190       7200
AGTGGTGGGA GGTGCCAGCC AGGGCTGGGA GTTGGACCCG AAAGTACGTG GCCTGGGCTG

      7210       7220       7230       7240       7250       7260
TACTTTCTTC CCACGTTGCC CCTTCAGAGC AGAAGCAGCC AGTTGCTCCT GAAGCCTTGA

      7270       7280       7290       7300       7310       7320
CCAGGGCTCC TGAGTCCAGA GCCTTGCTCA GGGCACTAGC GTGGGAGGAG GCTTCCGCAT

      7330       7340       7350       7360       7370       7380
CAGTACAGGG CATCAGCACC CGCCTCCTCA GCTGACCCAG CCCCGTGAGG ACCCAGGCCC

      7390       7400       7410       7420       7430       7440
AGCCCCCTGT CATCCCCACC CCCACCTTGC CAAGCCCCTG CCCCCAGGAG CAGGGCTGAG
```

FIG. 19 (8/15)

```
      7450       7460       7470       7480       7490       7500
AGCGAGGTGA TCTGGGTTCT AATCCAGAGT CTGCTGCTGA CATGTGCTGA GCCCCAGGCC

      7510       7520       7530       7540       7550       7560
CATTGGTTTA CTTGCCCCAG TATTGAGCGA GCATCCACTG GGTACCCGCC CAGTGCCGGT

      7570       7580       7590       7600       7610       7620
GCTGTGCCAG GGGCCGGGGC ACAGAATAAA GCAGACCCGT CCCTGCTCTT CTGGCATTCA

      7630       7640       7650       7660       7670       7680
CAGTCTTGTG GAAACTCCAG ACTGAAAGTG CCCTTAGAGA TTATCCAGAT CAGCCCCTCC

      7690       7700       7710       7720       7730       7740
TTGTAGCAAT GAAGAGACTG AGACCCACAG AGGGGATGAG TTTGATCCAA GAAACAGACA

      7750       7760       7770       7780       7790       7800
AGATTAAGAT GCATGTGTCT TGAACCTTTT CAGTGCTCTG GAACATACCG TCTGGCCGGA

      7810       7820       7830       7840       7850       7860
GTTGTCTGGG CTTTGGTTTT CCCATCCATG AAATGGGTAC AATAACAACA GCTATAGTGT

      7870       7880       7890       7900       7910       7920
ATGAGCCTCT GTGATAGATG CTGTACGCAC AGCACCTGAA CTCACATGAT AAACCACTGA

      7930       7940       7950       7960       7970       7980
GGTGAGCATT ATCTCCCATT ATCAAGGAGG ACCCTGGGGC TCAGAGAGGT TAAGCACGAT

      7990       8000       8010       8020       8030       8040
GCCAAGGCCA CACAGCCAGG GAAAGAAGAG TTGGAATTCA AACCCCGGGT GCCCTGTCTC

      8050       8060       8070       8080       8090       8100
ACACTAGCTT CCCCTGTGGA GGGTGCTGGT GTGTGCATGA TTGGAGGCCC TCACACAGTG

      8110       8120       8130       8140       8150       8160
TAAGTCTCAG GATCTGCAGC AAACTGGTCA GAATGCTCTG CCCTGGCCCA GGGAAGGAAA

      8170       8180       8190       8200       8210       8220
GAGGGGCAGA TGGAGTTTGC TTCGCTGTAA GGCCCCGGAG CTTTGTGTTC CTGCTGAGAA

      8230       8240       8250       8260       8270       8280
GCCTCAGAGT CGGGCAACAC TGGGTCTAAT TCCAGCTCCA CCCCTTGTAT TAATAGCTGG

      8290       8300       8310       8320       8330       8340
GCCTTAATCT CCTCATCTGT AAAATGGAGA GAATCGTCGC CTGTACTTCA TAAGGCTGCT

      8350       8360       8370       8380       8390       8400
GGAAGGATTA GCTAAAGCAA CCCAGCTACA GTGGCTGGCC TACAGTAGGT GCTTCATTAA

      8410       8420       8430       8440       8450       8460
TGCCCTTCCT TTTAGATGTG GAAATTCCTC TTTTTGTCCA AGTTTTCTTT TCCTCTTTGC

      8470       8480       8490       8500       8510       8520
TTACGGCACT GGGATTTTCT TTATTACTGT TTCTTTGAAG AGTCCGCTCT GTACTTGTGC
```

FIG. 19 (9/15)


```
      8530        8540        8550        8560        8570        8580
CCACGGCTAT GGTCAGTAAC CCCTTATGGA ATAAAACCCC TTTCCTGGCC AGGTGTGGTG

      8590        8600        8610        8620        8630        8640
GCTCATACCT GTAATCCCAG CACTCTGGGA GGCTGAGGCG GGAGGATCAC TTGAGCCCAG

      8650        8660        8670        8680        8690        8700
GAGTTCGAGA CCAGCCTGGG CAACACAGTG AGACCCCTGT CTCTACTAAA CATACAAACA

      8710        8720        8730        8740        8750        8760
ATTAGCCAGA TGTGGTGGTG CATACCTGTA GTCCCAGCTA CTCAGAAGGC TGAGATAGGA

      8770        8780        8790        8800        8810        8820
GGATCACCTG AGCCCAGGAG ATGAGGCCAC AGTGAGCTGT GATTGCACCA CTGCACTCCA

      8830        8840        8850        8860        8870        8880
GCCTGGGCAA CAGAGTGAGA CCCTACCTCA AAAAGAAAGC AACAACAGAA AACCTATTTC

      8890        8900        8910        8920        8930        8940
CCTATCCTAA TTGCACCTCC ATTCAAAGAG CTGCCCCTGC AAGAGTTAAC CAACTCCCTA

      8950        8960        8970        8980        8990        9000
GCCTCCCATG AGTTCTGAAA TCCTGCACCC AGGCCTGGTC CCAGTTGCCT AGCAACCGGG

      9010        9020        9030        9040        9050        9060
GGCTGCTCTG GGATGCAGTA GGTAAGCAGG GGAGGGAGAG GAAGAAAACA ACTTGGTCTG

      9070        9080        9090        9100        9110        9120
TCCACGACTC TAAATGTCAC TGAGAGATCA GTGCAGAGAA AGGCCTGTCA CCAGAGCCCA

      9130        9140        9150        9160        9170        9180
GGGCCCAATT TGCCTGGTGG TAGGGACAGC TGCCCTCAGG CCACCTGGGA GGTGGTTATC

      9190        9200        9210        9220        9230        9240
CCTCCTTTGA GTGGGCTTAC ATAACTACTT GGCATTTTTG CAAGGGACTT TAAGCTCACT

      9250        9260        9270        9280        9290        9300
CAGCAGTGAC ACCCCCCTCC GCCCACATGC ACATACATGT GTGGTACAGG GAGGACCCGG

      9310        9320        9330        9340        9350        9360
TGTGGGAGGC AGAGATGGGG TTCCAGCCAA CTGAAACTCC ATCATCTGCA TCTCCCGGCC

      9370        9380        9390        9400        9410        9420
TCTGACTGCC TCCCTCTGCC AAAGCGGGAA GATGAAAATG GTAACTGCTG GAATTTGTAT

      9430        9440        9450        9460        9470        9480
TTTGCAAAGA CTTTTCTCAT TTACTGCTGA ATATATTCCT CATCTCAGCC TCCACTCGCT

      9490        9500        9510        9520        9530        9540
GACACGCTAC CCACTGTCTC TCCCAGCATT CATCTCTACC TGAAATGATC TTGTTTACTT

      9550        9560        9570        9580        9590        9600
CTCTGTGTCT GTGTGCCTCG ACTCTCCCCC ACCGACTAGA AAGGTCCGTG AGAGCAAGGA
```

FIG. 19 (10/15)


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      9610       9620       9630       9640       9650       9660
GCAAGCCTGT CTTGTTTGAG GGCACTGGTT CTCATAGAGC CACAGGGAAT GATGCCCCTG

      9670       9680       9690       9700       9710       9720
GACTAAGCAG TGTGGGGTCT GCTGGCTTGC ACCTGTGCCC CCAGCTCCTA GCCAAAGACC

      9730       9740       9750       9760       9770       9780
AGACACATGT TGGGAACTCA ATACTTGTTT GTTTAATGAG TAGATGAACA AAAGCACTCA

      9790       9800       9810       9820       9830       9840
TGAAATAGGC AGTGCACGTA TCTTTATCAC CATTTGAAAG CTGAGGAAAC AGGCTTGGAG

      9850       9860       9870       9880       9890       9900
AGGGAAGCAA CTTGCCTGAC ACCCCAAATC ACAGAAGCAG CATATTTGGC CCAAGAACCT

      9910       9920       9930       9940       9950       9960
GGCTTCCTGT CTCCAAGGGG TCAGGTCCAG CTGGCATTGG CCTGTAGGCA TGTGAGTGTG

      9970       9980       9990      10000      10010      10020
GCAAGGTAGT CAGCAAAGAG CCTTTACTGC ATGTTGGGGT CAGAAGATCA GCAATAAGGA

     10030      10040      10050      10060      10070      10080
GGACAAAATC CTTGCCTGGA AGGAGCTTGT GTTCCAAAAA GAACAAGAGA CCACAGCATA

     10090      10100      10110      10120      10130      10140
TTCATTAATA AAGACACATT CAAACAGGGC CAAGTGCTCT GAAGCACCTC AGACAAAGCG

     10150      10160      10170      10180      10190      10200
ACAGGCTGCA AAATGACAGC GTTTGGGGGT CAGGAGACAG AAGGGTGCCT GCTTTAGGTG

     10210      10220      10230      10240      10250      10260
GTCGAAGAAG GCCTCTCTGG GGAGGTGGCA TTTGGTCTGA GACCTCAGGG CCAATGTGCT

     10270      10280      10290      10300      10310      10320
AGGAGCAGAG GAGCCTTGGG GAAGAATGGA GATGAGGTTG GACAGGATGA GACACGTGCC

     10330      10340      10350      10360      10370      10380
TTCTATGTCA ATGGCAAGGG AGTCATTGGA GCATGTGAAG CAGAGGATGC TCTACTTTTG

     10390      10400      10410      10420      10430      10440
CCCCAGAAAG ATCACTCTGG CTACAGTGCA GAGAAAGAAG AGAGTCAAGG AGGAAAGAAG

     10450      10460      10470      10480      10490      10500
GGCCTCATTA GGGGACTGTT GCAAAGCACA GGGAGGCACA ACCACAGCCA AGATCAGCAT

     10510      10520      10530      10540      10550      10560
GGTGACCAAT GGATGGAAGT GTCAGATGTC GCATGCTGTC GGTAGGTCAG GGCCGACAGG

     10570      10580      10590      10600      10610      10620
ACCTGTCGAT GGGTTCAGCG TGGGGTGTGA AGGAACACAG GCTGCACCCC AGCTCCTGGC

     10630      10640      10650      10660      10670      10680
CTGAGTGGCT GTAGATAGTG GCACCAAATA CTGAGCTCGT GAAGATGGGG GAGAGCTGAT
```

FIG. 19 (11/15)


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     10690      10700      10710      10720      10730      10740
GATGAAGACA GCAAGAGTTT GGTGTGAGTC ACCTTGAGTT TGAGACACGT GTCAGACATG

     10750      10760      10770      10780      10790      10800
TAAGGGGTAG GCAGGTGGAC ACGTGCTTAT TGAAGTCTGG AGCCAAGGGA GAGGTGTGGG

     10810      10820      10830      10840      10850      10860
CTGCAGCGGA GAAGTTGGGA GTATTCAGAG TTCTGACACT GACCAAGAAC ACCCCTCAGA

     10870      10880      10890      10900      10910      10920
GAATTCAGAG ACAACCAGGG CTGAGGCGAG GGGCTTAGAC TGGGGCCTGG GACAGCCACA

     10930      10940      10950      10960      10970      10980
GGCAGGAATG CAGACTTGCT GCCTCTTCTT ATTTGTGGAG ATGTAGTTCA TGCAGCAAGA

     10990      11000      11010      11020      11030      11040
AAGTCATTCC AAAGCCCTCC TTTCCTTTCT TCATGCCTCA GTTTCTCCAT TAGCACATTA

     11050      11060      11070      11080      11090      11100
AAAGATGCAA GATCTGGAGT TAAGCTTGTT TTTAAAAGGT GGCCTCCAAA GACGGTTTTT

     11110      11120      11130      11140      11150      11160
CTTGGCCTGG GGCTGTCTCA TCATCCAGGT CATGACAGGC CCGGTCCATG GTTGAGGAAT

     11170      11180      11190      11200      11210      11220
GCCACAGAAG TGACAGTCCA CTGCAAAAGA CTGCTGCTCC AGATCAGTTC TGGAAGGCCT

     11230      11240      11250      11260      11270      11280
GGCAATGGGG CAGGCCACTG AAGTAGAACT GGATGTCAGA TGCACGCATT AGAAAGGACA

     11290      11300      11310      11320      11330      11340
GGAAGACCAA ATGAGAAAGG GAGAGGGGGC AGGGAGAAAG GAAGGAGAGC TAGAGACTTG

     11350      11360      11370      11380      11390      11400
AGGCAAAGGA AACAAGAGAT GGAATAGAAG AAGACAGAGG ACCAGAAGAC AGTGAGACCA

     11410      11420      11430      11440      11450      11460
ACAGAAAGAG AGAGGGACGA GAAAGAAGGT GGCTGAGGAA GGTGAGAAAA GTGTTTCCAG

     11470      11480      11490      11500      11510      11520
GGCGACAGCA ACTGGACCAG GCCCTCTAGT TGGACAGTGA GGCTGGCTGG GGGGCCTGAG

     11530      11540      11550      11560      11570      11580
CTCAAGTAGC CCTCGTCCCC TGAGAGAGTG GGGGCTACCT GGGGAGCTGG GCTTGATGCA

     11590      11600      11610      11620      11630      11640
TCTGGAAGGA TCTTCACAGA GGCAGGAGGG GGAGTGGGAG GGCAGAGGGC ACCCAGGCGC

     11650      11660      11670      11680      11690      11700
TAGAACAGTG GGAGTGGCGG GACGCAAAAC CGGAGAGCCA GAGGAGTGAA CATCCCTGGC

     11710      11720      11730      11740      11750      11760
AGATTCCCCT GCGGCCGAGC AGGAGGGCAG GAAGCTCAGT GGTGTTGGCA CAACGTGAGA
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FIG. 19 (12/15)

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     11770      11780      11790      11800      11810      11820
AGTTCCAGGG AGGCGTGGGA GGACGGCTTC TGCAGGACGC AGACTTTGCA GAGGGAGAGT

     11830      11840      11850      11860      11870      11880
GGCAAACAGA CTGACTGCAG GCAGCTCTGC CGGCTCCACA GGGCGCTGCT TTTTCTCCAC

     11890      11900      11910      11920      11930      11940
GGTGGAGCTG GAGTGCATCA CCCTGAGAAC CAGCAGCAAG CCCCCACAGG GCACCTTCTG

     11950      11960      11970      11980      11990      12000
CGTGCCAGGC ACATCCGGAC CACTTGTCGG TAGACACCAG TGACCCTCAC CACCACCCCA

     12010      12020      12030      12040      12050      12060
GGAATGGGAC AGTGTCATGT GTTTCTGAAA TGACTAGGTT TTAGCACCAT TTCATAGATG

     12070      12080      12090      12100      12110      12120
AGGAAGCTGA AGCTAACTTG CCCAAGGTCA TAAACCGGGC GTCTGGTGGC CTCCCCTCCT

     12130      12140      12150      12160      12170      12180
CACTGCCAAC CCTGAGAGCG GACTAGGGTG GAGTTATCTG GAAAGAGGAA GCTGTACCTG

     12190      12200      12210      12220      12230      12240
AGAGCCCTAA ACACACATGC GCGCGCACGA CACACACACA CGCACAAACA CACAATGCAC

     12250      12260      12270      12280      12290      12300
GCACACACAT GCGCACGCAC ATACACACAC ATGCACACAT GGACACATAC CTGCACACAC

     12310      12320      12330      12340      12350      12360
AAGCATACAC ATGCACACAG GCACACGCAT GCACACACGC GCATGCACAC ACATGCACAC

     12370      12380      12390      12400      12410      12420
ACATGTGCAT GCACACAGTG CGACAGCTCT GATTAGTAGG TAAATAAAAG GTTCCCATCT

     12430      12440      12450      12460      12470      12480
AGTGGTGACT CGGCCAAAGT GCAGACACTG AACCCCAAAG GCCCATAGAG GCTTCATTCA

     12490      12500      12510      12520      12530      12540
TCCCTTCTCT TATTCTTCAT TCATGGATTC TATTGAGCAT CTGCTCTGTG CAGCATCTGT

     12550      12560      12570      12580      12590      12600
CCTGGATGCT GGGGATACTG TGATGACTTA GACAAGGTCT CAGCCGCACA CAGCTTATGC

     12610      12620      12630      12640      12650      12660
TTCTTTGAGG GGAGGCAGAC ACAAGCCAGG AAACCAATAA GAGAAGTTAA GTAAAAAGCA

     12670      12680      12690      12700      12710      12720
CAGTGAGTGA GACAAACGGG TACGGAGGAC ATGGCCAGAG AGAGCTTTAG TTCAGGTGGT

     12730      12740      12750      12760      12770      12780
CAGGGAGCAC CTCTCTGAGG AGGTGAAATT TGACCAAGCC TCAAACAGTG GCAGGGATCC

     12790      12800      12810      12820      12830      12840
CACTGCTTGC AGATCCTGGG GAGAAGCATT TTAGACAAAA AGAACAGCAA GTCCAAAGGC
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FIG. 19 (13/15)

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     12850      12860      12870      12880      12890      12900
CCAGAGACAA GACAGAGCAA GACCTGTGAC ATGAAACAGG CTGGTGTGCC CAGAGCAGGG

     12910      12920      12930      12940      12950      12960
AGGCTGGGAG AGTGGAGGGG GAGGGCGATG AGGGTGGAGA AGCTGGTGAG GGTGGCATCC

     12970      12980      12990      13000      13010      13020
CGGCAAGTGT GCCTGGCCAC GGAGGCCACG GAAGGATTCA GCATGTCTTT CCCGAATAGG

     13030      13040      13050      13060      13070      13080
AACCACACTG GGCTGTAACA GAGAGTGACG TACTCGGTAC GTTGAGAAGG TCCTGCTTAT

     13090      13100      13110      13120      13130      13140
TTCCTTCCGT GAAGGAGGAA GAGCTGCTGA TGACAGAGAT TGGCAGTGGC CAAAGACATA

     13150      13160      13170      13180      13190      13200
GAGAGAAGAG GGCAGAACAT GGGCTATTTT AAACACAGAG AAGATTAGCG GGACCCGCTG

     13210      13220      13230      13240      13250      13260
GCAGACCGGA CGTGAAATGT GGAAGGAGCG GGGGCAGCGA GGTCGGCTCC TAGTTTCCTG

     13270      13280      13290      13300      13310      13320
AGAATGTGGG TGAATCACGG GCTCACAGGC AGAGGGAGCA CTAGGATATC AAGGGTTCCC

     13330      13340      13350      13360      13370      13380
TTGTGAACGC CTCAAGTTGG AGATGCCTGA GACATCCAAG TGAGATGTCA AGCAGGCAGC

     13390      13400      13410      13420      13430      13440
TGGAAATAGG AGATGAGCTC TGGGAAAATG CTCCCATCAC CCTGGCCTGT GTGCTGCCTG

     13450      13460      13470      13480      13490      13500
GGCGCACCCA TTCAGGGCCC TCCACGCAGC CCACGCCCCT GCCTCCTGAT TCCTTCTAGG

     13510      13520      13530      13540      13550      13560
CTTCTCCAGC ACTCGTGGGA TGCCCAGATG TGATCAGGGA AGGGCTTGAG GATGCAGGGA

     13570      13580      13590      13600      13610      13620
AGCTGTGGCT GAGAGCCCTA AACACACACA TGCACACGCA CACACACATA CACAGGCACA

     13630      13640      13650      13660      13670      13680
TGCACACACG ACCATACACA CACACAAATG CACGCAGATG CACACAAATG CATATGCACG

     13690      13700      13710      13720      13730      13740
CACACAAATG CATATGCACA CACACACATG CACACATATG CATACACGTA TCCCTTTCAG

     13750      13760      13770      13780      13790      13800
TGGCTTTCCT TTCTGTCCTT AACCCTTGGC CCCTTACAGT GAGCTCCCAG TTCTCCCCAG

     13810      13820      13830      13840      13850      13860
CCTTAGAACC AAACCCTGGG GCTGGGCTGG GAGCCCCCAG TGACCCTCTG TGTCTCTGTA

     13870      13880      13890      13900      13910      13920
GGTGGATGCA CCCTTGGTCC TGGTGCCAGC TGCCACTGCA GGCTGAAGGC CTGTGAGTGT
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FIG. 19 (14/15)

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     13930      13940      13950      13960      13970      13980
GACAAGCAAT CCGTGCACTG CTTCAAAGAG AGCCTGCCCA CCTATGAGAA AAACTTCAAG
                    Exon 4

     13990      14000      14010      14020      14030      14040
CAGTTCTCCA GCCGGCCCAG GTGTGGCAGA CATAAGCCCT GGTGCTAGGG ACACCACAGG

     14050      14060      14070      14080      14090      14100
GTCCCTCTCA TCATCCAGCA TCCGCTCTAG TGTTGCTCTT CCAGGAAGCC TTCTCAGATC

     14110      14120      14130      14140      14150      14160
ATCCCCAACA GGCCCCTGTT CTTCCACTGG GAGGGAGGAC AAAATGTCTC CCGCAGGGCA

     14170      14180      14190      14200      14210      14220
GCTCACCCTT CAGCATTCTG ACCAAGGGGA CTCCCTGTCG TTCAGCATCA GAGGGCTGGA

     14230      14240      14250      14260      14270      14280
GAGCAGAAAT GGGAAAGATG AGATGCCTGC CCTGCAGGAG CTGGCATTCT GTGGAGTGGG

     14290      14300      14310      14320      14330      14340
GAGGACTACA AATGCATGGA TATAGAAGTA AGAGACACAT TAGACTGTAG TAAGTGCTAT

     14350      14360      14370      14380      14390      14400
GATGCAGTAA AACAAAGGGA CGGGATAGAG ATGCACCCAA CCCCACATCC CAGGGGTTTC

     14410      14420      14430      14440      14450      14460
CAGGAGGGGA GAAGCCCCAG GATCTACCCC AAACTCTCTC TTCACCCCCA CTGCAAACCG

     14470      14480      14490      14500      14510      14520
GGACACAGAG CAGACTTGAG CGCCAGGCCC ATGCCCAGCT CTAGCTGGCA ACAAAGCCAC

     14530      14540      14550      14560      14570      14580
CACTTTCCTT GCCCCTCTGC GTCCTCAGTT TTTATGATGT CATTCTTAGC TTTTCTTATC

     14590      14600      14610      14620      14630      14640
AAGAGGCAGA ATCTGTTTTC CCCATCCCAT GAATCTGAAC TGGTCTTGTG GCTTAGTTTG

     14650      14660      14670      14680      14690      14700
GTCAATAGAA TGTTGTGGGA GGGATGGTTT ACCAGTTTTG AGCTAGGCCT CAGGAGGTCT

     14710      14720      14730      14740      14750      14760
AGGGCATGTC TACTCTCTCT TAGGACAGCT GCCCCCACCC TGCAAAAAAG CCTGGGCTAG

     14770      14780      14790      14800      14810      14820
CCTGCTGGAG GATGAGAGCC CACCTGGATC AGTTGTCTCA GCTGATTTCA GACACGTGAG

     14830      14840      14850      14860      14870      14880
AGAGAGCTCA GCGAGACTCA GCTTGTAGCT GACTACAGAT GTGTGAGGGA ACCTGGCTGA

     14890      14900      14910      14920      14930      14940
GACCAAAACA ACTGTCCAGC TGAGCCCAGG CTAAACTGCC AACATGCAGA ATTGTGAGCT

     14950      14960      14970      14980      14990      15000
AAATAAAGGC TGCTGTTCTA AGTCACTGGG TTTTGGTATG GTTTGTTAGG CAGCCATAAC
```

FIG. 19 (15/15)


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     15010      15020      15030      15040      15050      15060
TAACAGGTGT AATTGGTCCT TATTCCCTTA TTCACTGAGA GTGATGGGTT CTCAGCCCTG

     15070      15080      15090      15100      15110      15120
AGCTGGACTT GGAGGCCATG GAAATGCAGT GGACATGGCC TTTGTTCCTT ACCTTGAAGC

     15130      15140      15150      15160      15170      15180
TGTGGAAGGA GGTCAAGTTC ATGGAATAAT GGAGAACACA CAGCTGTAAT CGTTTGCTTG

     15190      15200      15210      15220      15230      15240
TTCAGGGAAC ACACATTTAT TGAGCACTTG CTATGTGCCA GGCACAGTGC CAGGCAGTAG

     15250      15260      15270      15280      15290      15300
GGATCCAGAT ATTTAAAGAA AACAAACAAA AATCAGGTCC AAAACTCCTG GGGAGAATGC

     15310      15320
TGAGAGTGGT ATCAGCTTTT AGGAATTC
```

Fig. 20 Diagram of Vector to Express Dicistronic mRNA

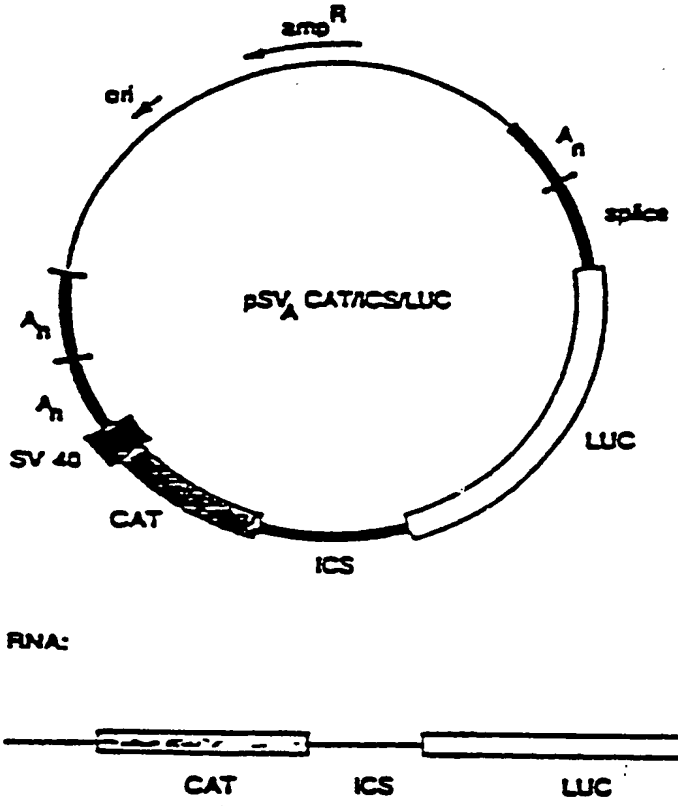

Fig. 21

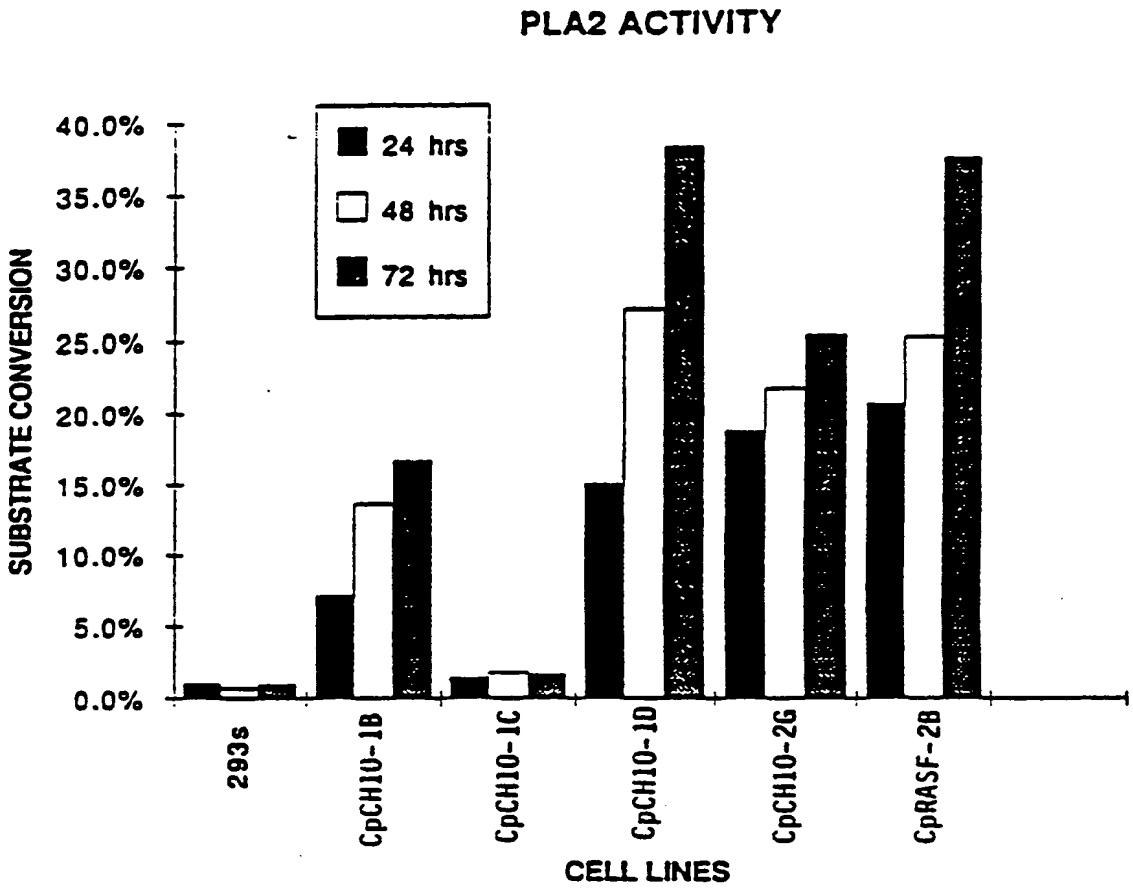

FIG. 22

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                  1                                                  50
Human Type I      AVWQFRKMIKCVIPGSDPFLEYNNYGCYCGLGGSGTPVDELDKCCQTHDN
Human Type II     NLVNFHRMIK-LTTGKEAALSYGFYGCHCGVGGRGSPKDATDRCCVTHDC
HPLA2-10          GLLDLKSMIE-KVTGKNALTNYGFYGCYCGWGGRGTPKDGTDWCCWAHDH
                           *                 **  ** *          * **  **

                 51                                                  100
Human Type I      CYDQAKKLDSCKFLLDNPYTHTYSYSCSGSAITCSSKNKECEAFICNCDR
Human Type II     CYKRLEKR-GC------GTKFLSYKFSNSGSRITC-AKQDSCRSQLCECDK
HPLA2-10          CYGRLEEK-GC------NIRTQSYKYRFAWGVVTC-EPGPFCHVNLCACDR
                  **                        *          *           *

               101                                  133
Human Type I      NAAICFSKAP--YNKAHKNLDTKKYCQS
Human Type II     AAATCFARNKTTYNK-KYQYYSNKHCRGSTPRC
HPLA2-10          KLVYCLKRNLRSYNP-QYQYFPNILCS
                       *       *
```

Alignment of amino acid sequences of human type I, II and $HPLA_2$-10 PLA_2. Asterisks denote eighteen residues that have been conserved among all active PLA_2 sequences. Underscored residues denote the amino acid COOH-terminal extensions.

Top line is SEQ ID NO:38:;
Middle line is SEQ ID NO:39:;
Bottom line is SEQ ID NO:40:.

FIG. 23

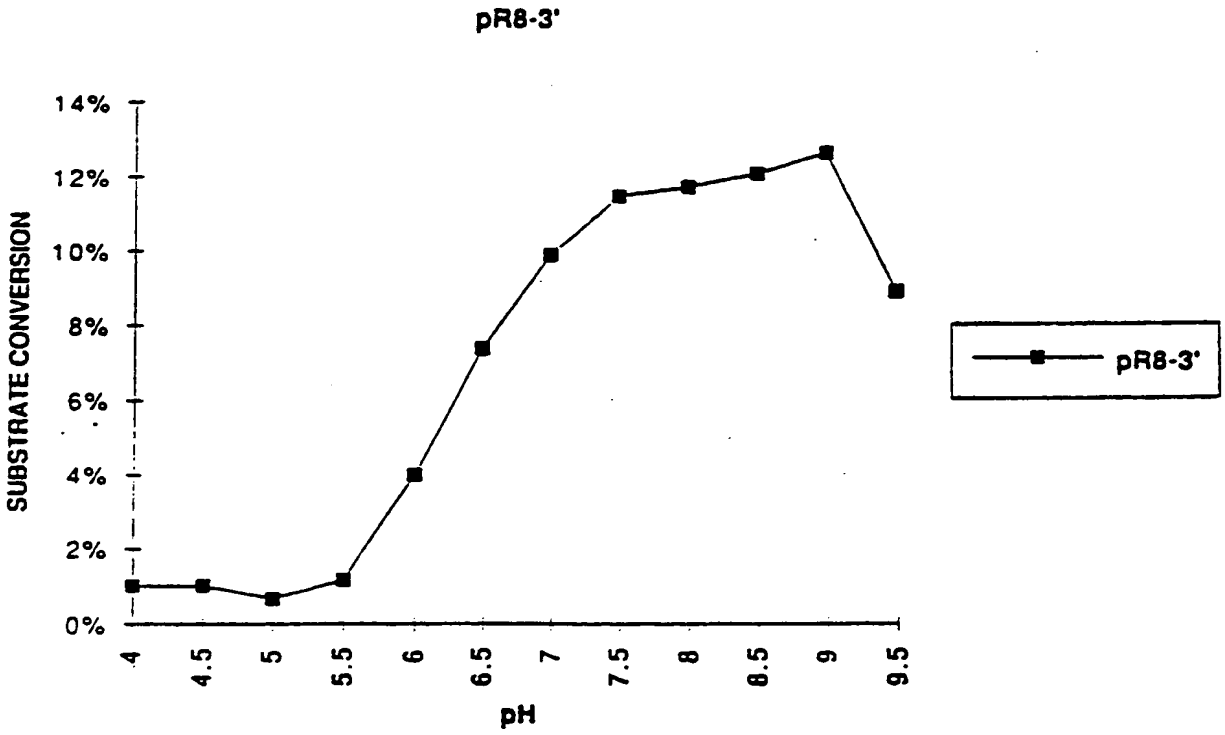


FIG. 24

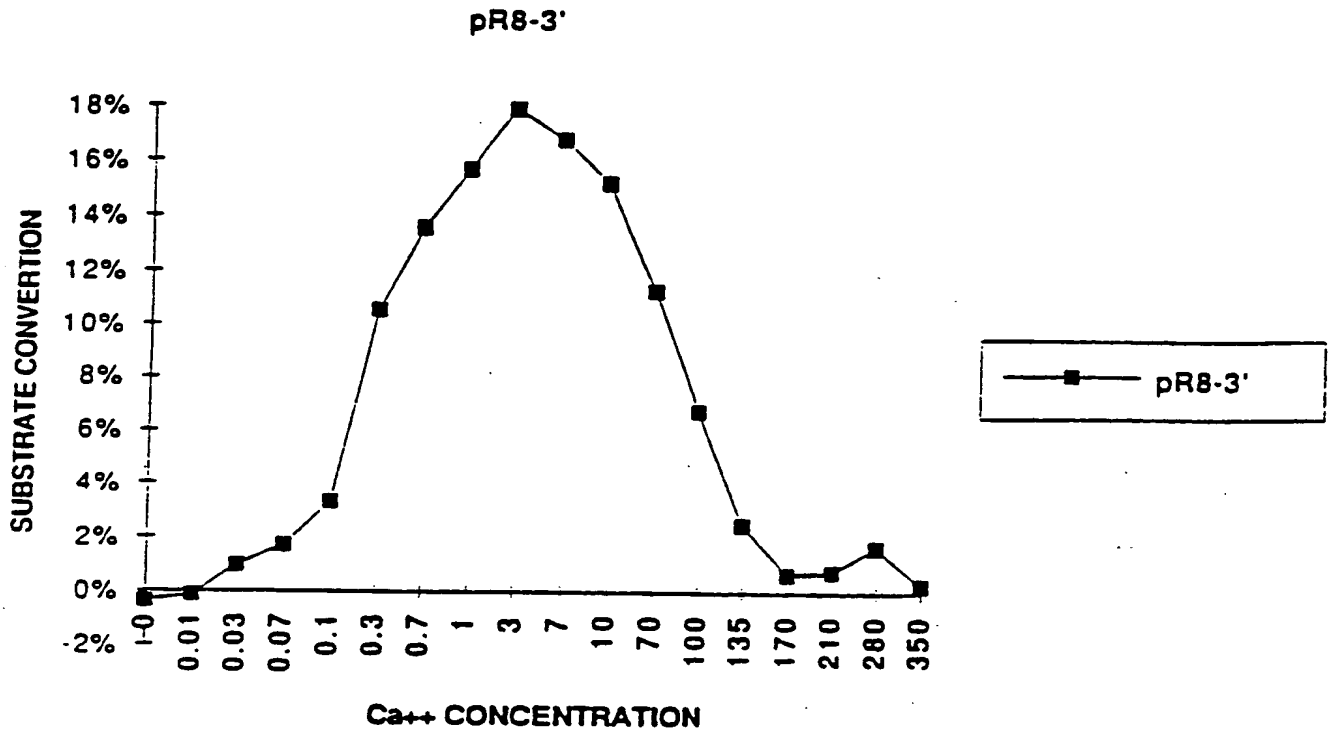


FIG. 25

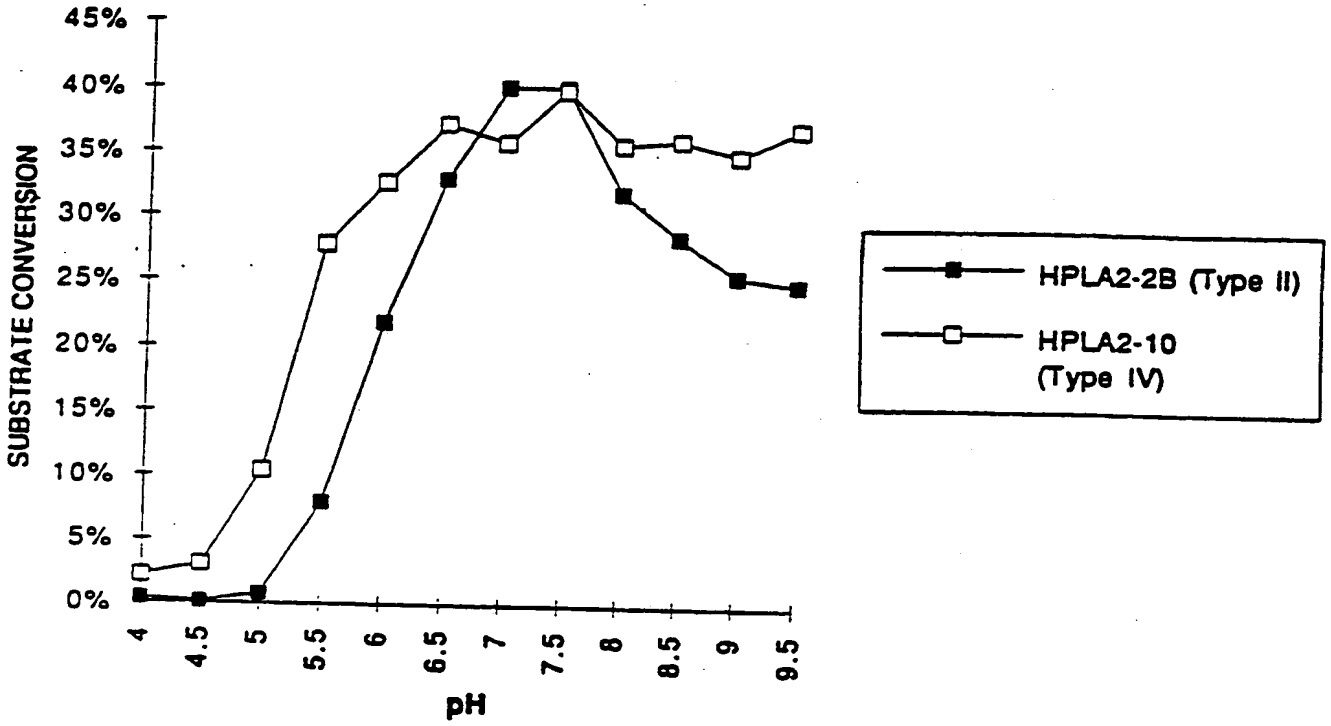


FIG. 26

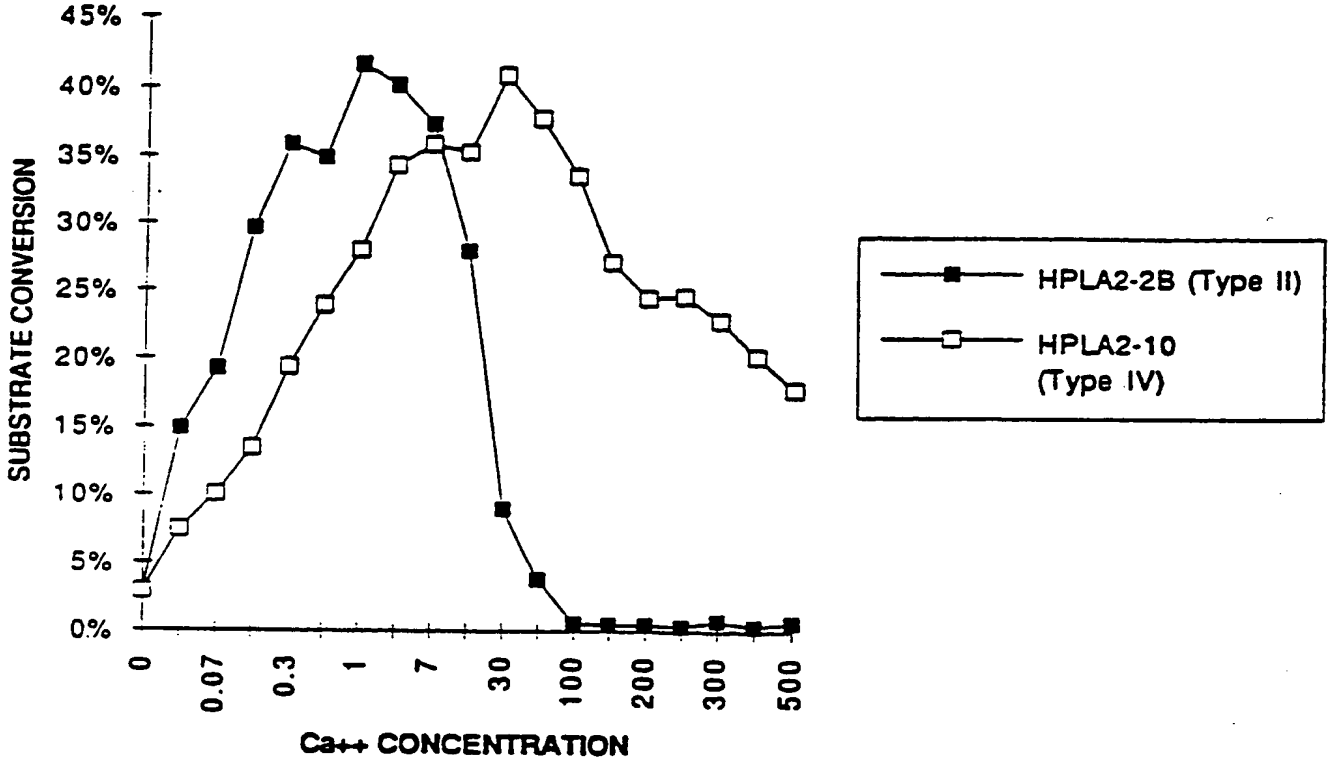
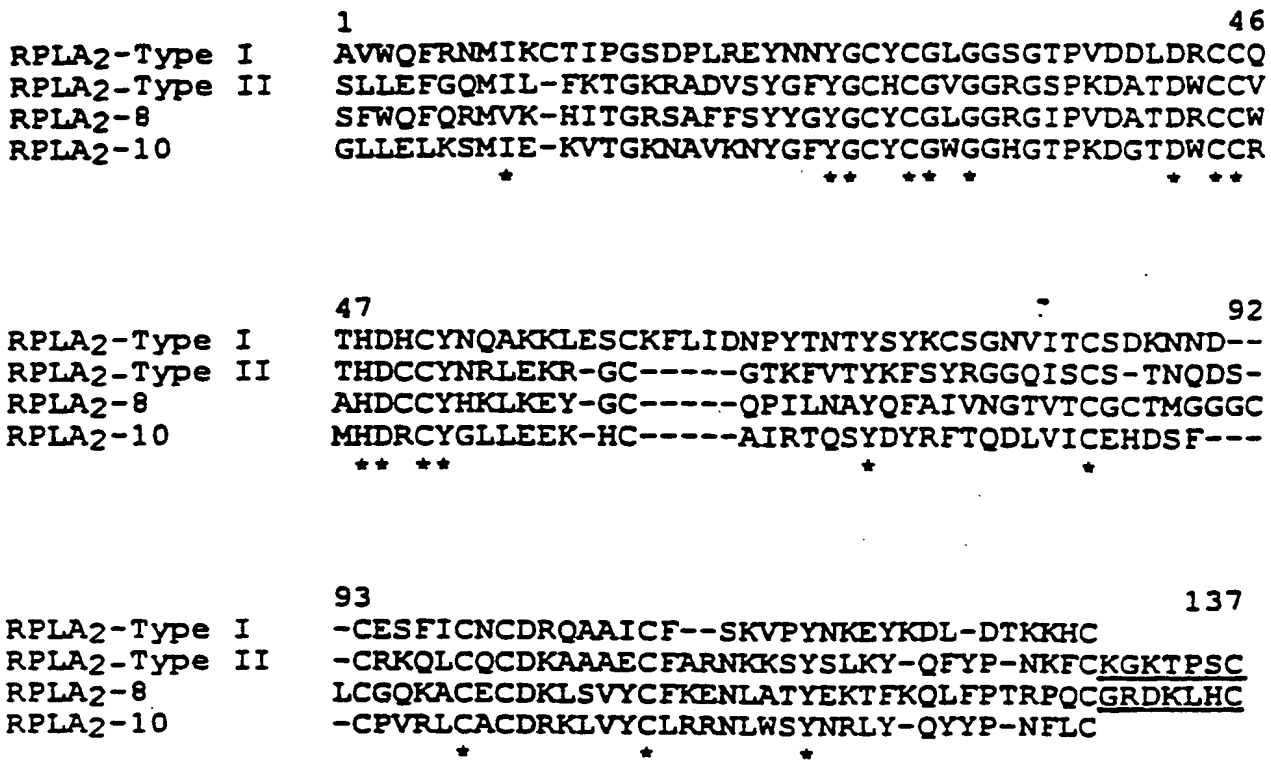


FIG. 27



Alignment of amino acid sequences of rat Type I, II, $RPLA_2$-8 and $RPLA_2$-10 PLA_2s. Asterisks denote eighteen residues that have been conserved among all active PLA_2 sequences. Underscored residues denote the amino acid COOH-terminal extensions.

$RPLA_2$-Type I sequence shown corresponds to SEQ ID NO: 41:; $RPLA_2$-Type II sequence shown corresponds to SEQ ID NO:42:; $RPLA_2$-8 sequence shown corresponds to SEQ ID NO:43:; $RPLA_2$-10 sequence shown corresponds to SEQ ID NO:44:.

INTERNATIONAL SEARCH REPORT

International application No.
US94/07926

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :435/69.1, 172.1, 172.3, 240.2, 320.1; 514/44; 530/350; 536/23.1, 23.5, 24.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 172.1, 172.3, 240.2, 320.1; 514/44; 530/350; 536/23.1, 23.5, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, BIOSIS, EMBASE, MEDLINE, DERWENT BIOTECHNOLOGY ABSTRACTS
phospholipase A2, gene, cDNA, type III, type IV, 14 kD, cloning

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Gene, Volume 93, issued 1990, T. Deng et al, "A novel expression vector for high-level synthesis and secretion of foreign proteins in Escherichia coli: overproduction of bovine pancreatic phospholipase A_2", pages 229-234, see the entire document.	41-52, 57-58, 60-61
A	Journal of Cellular Biochemistry, Volume 39, issued 1989, J.J. Seilhamer et al, "Novel Gene Exon Homologous to Pancreatic Phospholipase A_2: Sequence and Chromosomal Mapping of Both Human Genes", pages 23-33.	23-40, 53-56, 69-70, 75-84

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 OCTOBER 1994	Date of mailing of the international search report 24 OCT 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRUCE CAMPELL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/07926

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, f the relevant passages	Relevant to claim No.
Y	Biochimica et Biophysica Acta, Volume 1089, issued 1991, A.C.A.P.A. Bekkers et al, "The use of genetic engineering to obtain efficient production of porcine pancreatic phospholipase A_2 by Saccharomyces cerevisiae", pages 345-351, see the entire document.	41-52, 57-58, 60-61
A, P	Biochemical Pharmacology, Volume 48, No. 1, issued 1994, A.B. Mukherjee et al, "Phospholipase A_2 Enzymes: Regulation and Physiological Role", pages 1-10.	1-84
Y	Critical Reviews in Biotechnology, Volume 12, No. 4, issued August 1992, N-S. Yang, "Gene Transfer into Mammalian Somatic Cells In Vivo", pages 335-356, see the entire document.	59, 62-68

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/07926

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/07926

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A01N 43/04; A61K 31/70; C07H 17/00; C07K 3/00, 13/00, 15/00, 17/00; C12N 5/00, 15/00; C12P 21/06

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-22 and 71-74, drawn to PLA2 proteins.
Group II, claim(s) 23-40, 53-56, 69-70 and 75-84, drawn to nucleotide sequences encoding PLA2 proteins.
Group III, claim(s) 41-52, 57-58 and 60-61, drawn to expression vectors, host cells, and methods of making PLA2 proteins.
Group IV, claims 59 and 62-68, drawn to methods for gene therapy.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Group I is distinct from each of II and IV because the proteins of I are not required for the nucleotide sequences and gene therapy methods of II and IV, and the compositions and methods of II and IV are not required for production of the proteins of I.
Groups I and III are distinct, each from the other, because the proteins of I can be produced without the vectors, cells and methods of III. The protein can be isolated from tissues, or produced synthetically. Furthermore, the proteins of I are not required for the compositions and methods of III.
Group II is distinct from each of III and IV, because the nucleotide sequences of II can be used for several different purposes. Besides the methods of III and IV, the sequences of II can be used as hybridization probes for isolation of related genes.
Groups III and IV are distinct, each from the other, because the vectors, cells and methods of III are not necessary for the methods of claims 62-68. The method of claim 59 requires reagents and procedures not required by the methods of III, and the methods are not obvious variants. Furthermore, the methods of IV are not required for the production or use of the compositionsand methods of III.
Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.